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The Oxidation of Glucose in Aqueous Solution by Oxygen

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THE OXIDATION OF GLUCOSE IN AQUEOUS SOLUTION BY OXYGEN

A thesis submitted by

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SUMMARY

The oxidation of D-glucose in aqueous solution by molecular oxygen was studied over the temperature range from 110 to 140°C. The solutions were prepared from highly purified water and the reactions were conducted in a container fitted with a Teflon insert to avoid metal ion catalysis.

Kinetic data were obtained by following the amount of unreacted glucose with time at each reaction temperature. The reaction was well described as being of order 1.5 with respect to the glucose concentration up to approximately 35-40% reaction. The observed order is consistent with a kinetic scheme in which the reaction is initiated by the direct interaction of oxygen with glucose. The energy of activation in the temperature range studied was 26.8 kcal./mole. This value is comparable with the activation energies of other oxidations which are initiated directly by molecular oxygen. The phenomenon of autocatalysis was not observed.

At each temperature, the solutions were observed to darken with increasing reaction time, progressing from colorless through yellow to amber. At the longer reaction times, a dark brown, water-insoluble material was also found in the solutions.

Under the reaction conditions used in this work, there were two competing types of reactions; the oxidation of glucose and its dehydration to 5-hydroxymethylfurfural (HMF). The latter compound is readily converted by oxygen or acids (both of which are present) to darkly colored polymeric materials. Arabinose, a product from the oxidation of glucose, is a precursor of furfural. Furfural also contributes to the formation of the insoluble material.

The formation of HMF is thought to account for the deviation of the kinetic data at the higher degrees of reaction. The presence of HMF and furfural was indicated by the ultraviolet spectra of the reaction solutions. HMF was further identified by the isolation of its 2,4-dinitrophenylhydrazone.

Gluconic acid, arabinose, and arabonic acid were identified as nonvolatile oxidation products of glucose. Additionally, evidence was obtained for the presence of 2-ketogluconic acid, erythrose, and erythronic acid.

The formation of gluconic acid, arabinose, and arabonic acid with time was studied. From the data it was concluded that gluconic acid was the primary oxidation product of glucose. This conclusion is in accord with theoretical considerations regarding the position of initial attack.

Arabinose and arabonic acid are secondary oxidation products. Arabinose is formed from gluconic acid. Arabonic acid may be produced from arabinose in an analogous manner to the formation of gluconic acid from glucose. The data indicate that it is also formed from gluconic acid.

Glucuronic acid, xylose, and glucosone were not detected among the reaction products. This information further substantiates the conclusion that the position of attack by oxygen is at the anomeric carbon atom.

The only one-carbon fragment identified was carbon dioxide. Analyses were performed for formaldehyde and formic acid. It was concluded from this work that formaldehyde was not a product. Additionally, evidence was obtained which indicated that formic acid was not an oxidation product of glucose. However, it may have been produced during the degradation and subsequent polymerization of HMF and furfural to the water-insoluble material noted at the longer reaction times.

The discrepancy between the amount of volatile carbon produced and that accounted for as carbon dioxide could not be resolved. The difficulty was attributed, at least in part, to the experimental equipment. From considerations of the reaction products identified and of those shown to be absent, it was concluded that carbon dioxide was the most probable volatile product.

A mechanism for the oxidation of glucose in aqueous solution is proposed. The oxidation scheme involves the one-carbon, stepwise degradation of glucose by a free-radical chain process initiated by molecular oxygen at the anomeric carbon-hydrogen bond. The initial product of the reaction is a hydroperoxide which undergoes further reaction to yield glucono-1,5-lactone. Evidence has been obtained for the presence of peroxide compounds among the reaction products.

Experimentally, D-glucose uniformly labeled with carbon-14 was used as the starting material to facilitate the analysis of the reaction products. The primary analytical technique was isotope dilution. Ultraviolet spectrophotometry and descending paper chromatography were also employed.

INTRODUCTION

The reaction* of molecular oxygen with many types of organic compounds is of economic importance in several industries among which are the petroleum, rubber, textile, and paper industries. Its effects may be beneficial or adverse depending upon the conditions under which the reaction occurs.

In the drying oil industry, molecular oxygen is advantageously employed for the manufacture of blown oils and for the production of various oxidation and polymerization products (1). A wide variety of organic chemicals are manufactured in quantity by the controlled reaction of hydrocarbons with oxygen. The process of film formation in applied protective coatings is yet another example of this type of reaction. The adverse effects of the reaction of molecular oxygen with organic compounds are frequently observed with rubber, plastics, gasolines, and fuel oils. Rancidification and other forms of oxidative deterioration of fats and fat-containing materials are also attributable to the reaction of these compounds with molecular oxygen.

In areas more closely related to the subject of interest in this thesis, several examples of the importance of oxygen as an oxidizing agent may be cited. The aging (oxidation) of alkali cellulose is an important step in the manufacture of viscose in the rayon industry (2-3). Mehltretter, et al. (4) and others (5-7) have obtained patents for the preparation of aldonic acids and aldouronic acids by the catalytic oxidation of hexoses and their derivatives with oxygen. Additionally, Gillaspie (8) has proposed the use of molecular oxygen in a simple, low-cost process for the partial bleaching of chemical wood pulps or as the first stage of a multistage bleaching sequence. The detrimental effects of oxygen are observed in the aging (natural or accelerated) of paper (9).

*The reactions to which reference is made occur at temperatures less than those at which combustion takes place.

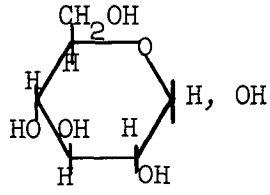
In the past several decades considerable work has been undertaken to elucidate the mechanisms by which saturated and unsaturated hydrocarbons react with molecular oxygen. Other organic compounds such as alcohols, aldehydes, and ethers have received less attention. A comprehensive review of much of this work is presented by Lundberg (10).

Investigations of the interaction of molecular oxygen with carbohydrates are not numerous. The areas which have been most heavily studied due to their economic importance are the aging of alkali cellulose, the thermally induced degradation of cellulose, and the effects of ionizing and ultraviolet radiation on cellulose in the presence of oxygen. These studies have been undertaken primarily to determine the gross changes in the physical and chemical properties of the cellulosic species. The heterogeneous nature of these reactions has often precluded an examination of the reaction products and the pathways by which they are formed.

In order to circumvent the difficulties inherent in the study of heterogeneous reactions of this type, recent investigators have utilized model compound studies in homogeneous systems. This approach has been used with considerable success by Phillips, et al. (11-22) and Beelik and Hamilton (23). They investigated the effects of gamma-ray and ultraviolet radiation on mono- and disaccharides and their derivatives. Church (24) has studied the thermally induced oxidation by molecular oxygen of methyl glycopyranosides.

As far as could be ascertained from a review of the literature, the oxidation by molecular oxygen of simple carbohydrates in neutral aqueous solutions has not been studied. This thesis was undertaken to provide basic information concerning the nature of the reaction products and the manner in which they are formed. Of necessity the reaction conditions are only an approximation to those found in the thermal degradation of cellulose or in the aging of paper. However, the results of such studies may one day prove useful in controlling the above phenomena.

The model compound used in this work was D-glucose, which was uniformly labeled with carbon-14 to facilitate the identification of the reaction products. The Haworth formula is shown below.



D-Glucose

The crystalline starting material was the alpha anomer, because it could be purified in high yield with little loss of the relatively expensive labeled glucose.

GENERAL FEATURES OF FREE-RADICAL OXIDATIONS

The oxygen molecule is known to be diradical in nature (i.e., it possesses two unpaired electrons). The majority of organic oxidation reactions in which molecular oxygen is the oxidant have been shown to be free-radical chain reactions (25-27). In all chain reactions there are at least three steps (28) which are most commonly described as:

1. Initiation: the introduction of free radicals into the system.
2. Propagation: the shifting of active centers to new sites.
3. Termination: the radical is destroyed or inactivated.

Many autoxidation* reactions of hydrocarbons have been well established as being free-radical chain processes (29-30). Schematically, the oxidation may be represented by the following reaction series (31-33) where the symbol R:H is used to represent the substance with its labile hydrogen atom undergoing oxidation.

1. Initiation:

$$R{:}H + O_2 \rightarrow R\cdot + HOO\cdot \qquad (1)$$

2. Propagation:

$$R\cdot + O_2 \rightarrow ROO\cdot \qquad (2)$$

$$ROO\cdot + R{:}H \rightarrow ROOH + R\cdot \qquad (3)$$

3. Termination:

$$2R\cdot \rightarrow \text{Inactive products} \qquad (4)$$

$$R\cdot + ROO\cdot \rightarrow \text{Inactive products} \qquad (5)$$

$$2ROO\cdot \rightarrow \text{Inactive products} \qquad (6)$$

It is seen from Equation (3) that the initial oxidation product is a hydroperoxide. Some free-radical reactions exhibit the phenomenon of autocatalysis due to the instability of the hydroperoxide formed initially.

*The term autoxidation is usually defined as the slow, spontaneous reaction of a compound with molecular oxygen at or near room temperature.

4. Autocatalysis:

$$2ROOH \rightarrow RO\cdot + ROO\cdot + H_2O \quad (7)$$

$$RO\cdot + R{:}H \rightarrow ROH + R\cdot \quad (8)$$

$$ROO\cdot + R{:}H \rightarrow ROOH + R\cdot \quad (9)$$

INITIATION

The reaction represented by Equation (1) illustrates the initiation of a free-radical chain reaction by the direct interaction of the diradical oxygen molecule with an organic compound. There appears to be some controversy regarding this initial reaction.

The autoxidation of olefins has been studied widely. From considerations of bond dissociation energies, Uri (34) has deduced that the initiation reaction shown by Equation (1) is endothermic, and therefore, thermodynamically unfavorable and of little practical significance. He is of the opinion that the initial attack by oxygen requires traces of heavy metal ions which act as catalysts in a series of electron transfer reactions or that initiation is caused by the decomposition of hydroperoxides by heavy metal ions to yield free radicals. In contrast to Uri, Bolland and Gee (35) have presented a thermodynamic argument based on bond dissociation and resonance energies to show that the direct initiation of the reaction by molecular oxygen is feasible. According to Russell (26), oxygen reacts directly with hydrocarbons at high temperatures to produce free radicals which lead to oxidation via a free-radical chain process. At lower temperatures (25-80°C.), such initiation reactions may occur but are difficult to detect, because initiation may also be catalyzed by trace amounts of hydroperoxides or metal ions present in the system. This view has also been expressed by others (33,36).

PROPAGATION

The reactions in the propagation sequence represented by Equations (2) and (3) are thought to occur rapidly, particularly the addition of oxygen to an alkyl radical since this is essentially a radical coupling reaction (26,37). The addition of molecular oxygen to a polystyrenyl radical has been shown to occur 10^6 times faster than the addition of a monomeric styrene unit even though styrene is extremely reactive towards free radicals (37). In some instances the alkyl radical may react with oxygen to form an unsaturated compound:

$$H\text{-}\overset{|}{C}\text{-}\overset{|}{C}\cdot + O_2 \rightarrow \overset{|}{C}=\overset{|}{C} + HOO\cdot \qquad (10).$$

Since the peroxy radical is stabilized by resonance, the formation of an unsaturated compound can compete with the formation of a peroxy radical only when the former has unusual stability due to conjugation (26). Occasionally, the free radical formed in Equation (1) may be so highly stabilized by resonance that it reacts slowly if at all with molecular oxygen (37). The most frequently observed reaction of a peroxy radical is the abstraction of a labile hydrogen atom, commonly found in the alpha position to a double bond, an aromatic ring, or an ether linkage. The hydrogen atom attached to the carbon atom of an aldehydic carbonyl group is labile also.

The rapidly proceeding propagation sequence of Equations (2) and (3) assumes a high degree of mobility of the reactants in order that the free radical produced in Equation (2) can abstract a hydrogen atom from another reactive center as in Equation (3). The mobility of a reactive point on a long chain is very much less than that on a small molecule, and consequently its potential sphere of reaction is greatly reduced (38). Although the precise effect of reduced chain mobility on the rapidity of a reaction is difficult to estimate, reduced mobility does

retard the separation of free radicals produced in close proximity and tends to eliminate the possibility of a reaction between two chains far apart initially.

TERMINATION

The generally accepted reaction by which termination occurs is illustrated by Equation (6) and involves the combination of two peroxy radicals to yield inactive products (33-34,37,39-42). Termination reactions such as the combination of two R· radicals or one R· radical and one ROO· radical are important at low oxygen concentrations only, since the radical ROO· is present in much higher concentrations than R· due to the rapidity of the reaction in Equation (2). For most olefin oxidations the termination reactions represented by Equations (4) and (5) have been found to be negligible above an oxygen pressure of 0.13 atm. (33-34). Generally, termination reactions account for only a minor fraction of the total primary reaction products, because the propagation chains are usually quite long.

AUTOCATALYSIS

The phenomenon of autocatalysis observed during some reactions results from the decomposition of the hydroperoxide initially formed into free-radical products which may then also initiate reaction chains. The decomposition reaction is generally thought to be bimolecular in nature.

Some organic hydroperoxides are unstable and are decomposed by heat, light, and/or transition metal ions (43). The action of heat or light may cause homolytic fission of the oxygen-oxygen bond thereby producing two free radicals as illustrated in Equation (7). The decomposition of a hydroperoxide by a transition metal ion such as iron, cobalt, or manganese produces one free radical and is often

represented as follows (32,37,44):

$$ROOH + M^{++} \rightarrow RO\cdot + OH^{-} + M^{+++} \quad (11)$$

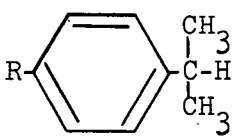
$$ROOH + M^{++} \rightarrow RO^{-} + HO\cdot + M^{+++} \quad (12).$$

INITIAL SITE OF ATTACK BY FREE RADICALS

The majority of organic oxidation reactions involve the rupture of carbon-carbon or carbon-hydrogen bonds. In a free-radical reaction, homolytic cleavage of a carbon-hydrogen bond occurs by free-radical abstraction of a labile hydrogen atom. The abstracting species may be the strongly electronegative oxygen molecule or another free radical.

There appear to be two main factors which determine the susceptibility of a carbon-hydrogen bond to attack. They are the availability of electrons at the bond to be ruptured and the stability of the resultant radical after the removal of a hydrogen atom (37). In cases where attack at a particular carbon atom might be inhibited due to the electron-withdrawing effect of a neighboring atom but where the intermediate radical would be stabilized by resonance, the resonance effect appears to dominate generally. For example, this type of situation is encountered in the oxidation of alcohols, aldehydes, and ethers. The effects of these factors are explicitly or implicitly presented in the following paragraphs wherein the oxidation of various organic compounds by molecular oxygen is discussed.

The effect of electron density at the point of attack on the ease of removal of a hydrogen atom was studied by Russell (37,45) during the oxidation of para-substituted cumenes:



The tertiary hydrogen atom was most easily removed (i.e., the rate of reaction was greatest) when the group R was an electron releaser, such as a tertiary butyl group. An electron-withdrawing group, such as the nitro group, caused the greatest reduction in the rate of reaction. Similar conclusions were drawn by Walling and McElhill (46) from their study of the reactivities of substituted benzaldehydes.

In the autoxidation of olefins, it is well known that hydrogen atom abstraction occurs at the carbon atom alpha to the double bond since the resultant radical may be stabilized by resonance as illustrated (36-37,47):

$$R\text{-}CH_2\text{-}CH{=}CH\text{-}R' + In\cdot \rightarrow \left(\begin{matrix} R\text{-}\dot{C}H\text{-}CH{=}CH\text{-}R' \\ \updownarrow \\ R\text{-}CH{=}CH\text{-}\dot{C}H\text{-}R' \end{matrix} \right) + In{:}H \qquad (13)$$

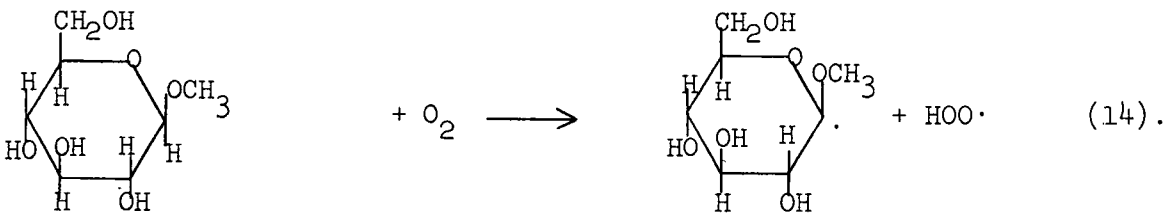
where In· represents a generalized initiating radical. Russell (37) has shown that a linoleate compound is ten times more reactive than an oleate compound and one hundred times more reactive than a stearate compound. These compounds contain two double bonds separated by a methylene group, one double bond, and no double bonds, respectively. Hence, the possibilities for resonance stabilization of the linoleate compound after the removal of a hydrogen atom exceed those of the other two compounds.

Ross, et al. (48) studied the autoxidation of methyl oleate and were able to demonstrate the effect of resonance stabilization by isolating and identifying the four isomeric hydroperoxides which may be formed by abstraction of a hydrogen atom from the carbon atoms which are alpha to the double bond.

When ethers and primary or secondary alcohols are oxidized, the initial point of attack is at a carbon atom in the alpha position to the oxygen atom of the ether linkage or the hydroxyl group, respectively (37,49). In this manner the adjacent oxygen atom may contribute to the resonance stabilization of the radical formed

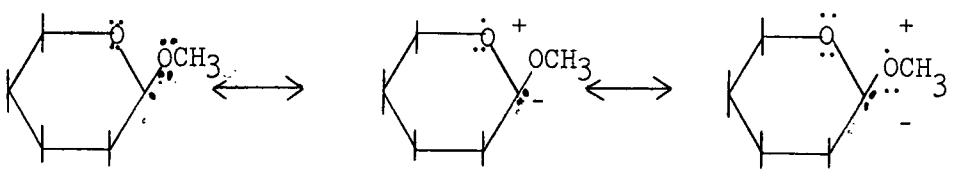
after the hydrogen atom has been removed. McBurney (50) calculated the theoretical loss of ethoxy groups and the amount of hydroperoxide formation based on this type of reaction mechanism for the oxidation of ethyl cellulose. The agreement between the theoretical values and those determined experimentally was very good.

Aldehydes and acetals undergo oxidation at the carbonyl carbon atom and the carbon atom bonded to the two oxygen atoms of the acetal linkage, respectively (36-37). Both types of bond arrangements are found in carbohydrates and carbohydrate derivatives. Church (24) concluded that the attack of molecular oxygen on crystalline methyl-β-D-glucopyranoside (having an acetal bond structure) in the molten state occurred almost exclusively at the anomeric carbon-hydrogen bond:



(14).

The free radical produced may be stabilized by the following resonance structures:



(15).

KINETICS OF OXIDATIONS BY MOLECULAR OXYGEN

The kinetic theories used for the development of rate expressions for reactions in which a free-radical chain mechanism is operative are based on the following postulates:

1. The concentration of chain carriers reaches a stationary value.

2. The number of initiation and termination reactions are small compared to the number of propagation reactions.

Since the oxygen pressure was relatively high in the present work, a third postulate is useful:

3. The termination reactions involve only the combination of two peroxy (ROO·) radicals.

In the succeeding section the rate expression for reactions initiated directly by molecular oxygen is developed. Since some oxidations in which oxygen is the oxidant are observed to be autocatalytic, a rate equation for this situation is also presented.

KINETICS OF OXYGEN-INITIATED REACTIONS

The following derivation for the oxidation of an organic compound in which the reaction is initiated by molecular oxygen is adapted from the work of Cooper and Melville (40).

The free-radical chain mechanism operative during the liquid-phase, homogeneous, oxygen-initiated oxidation of an organic compound, R:H, may be represented by the following equations if the reaction is nonautocatalytic:

Reaction	Rate	
$R{:}H + O_2 \rightarrow R\cdot + HOO\cdot$	$k_i[R{:}H][O_2]$	(16)
$R{:}H + .OOH \rightarrow R. + H_2O_2$	$k_r[R{:}H][.OOH]$	(17)
$R\cdot + O_2 \rightarrow ROO\cdot$	Fast	(18)
$ROO\cdot + R{:}H \rightarrow ROOH + R\cdot$	$k_p[ROO\cdot][R{:}H]$	(19)
$2ROO\cdot \rightarrow$ Inactive products	$k_t[ROO\cdot]^2$	(20).

Since the concentration of free radicals in the system reaches a constant value, the rate of free-radical production may be equated to the rate of free-radical

removal. Equations (16) and (20) are the only ones which result in the net production or removal of free radicals; therefore, their rates must be equal:

$$k_i[R:H][O_2] = k_t[ROO\cdot]^2 \qquad (21).$$

Solving for [ROO·]:

$$[ROO\cdot] = (k_i/k_t)^{0.5}[R:H]^{0.5}[O_2]^{0.5} \qquad (22).$$

If the number of initiation reactions is small compared with the number of propagation reactions, most of the compound R:H will be consumed in the reaction given by Equation (19), and we may write:

$$-d[R:H]/dt = k_p[ROO\cdot][R:H] \qquad (23).$$

Substituting the value of [ROO·] from Equation (22):

$$-d[R:H]/dt = k_p(k_i/k_t)^{0.5}[R:H]^{1.5}[O_2]^{0.5} \qquad (24).$$

It is seen that the rate of reaction in the oxygen-initiated oxidation of an organic compound is of order 1.5 with respect to the concentration of the compound and of order 0.5 with respect to the concentration of oxygen (in the solution phase) for a nonautocatalytic free-radical chain reaction.

KINETICS OF AUTOCATALYTIC REACTIONS

The phenomenon of autocatalysis observed during some oxidations results from the decomposition of the hydroperoxide initially formed into free-radical products which may then initiate reaction chains. The decomposition reaction is thought to be bimolecular in nature. If it is assumed that the initiation of the reaction occurs in this manner, the following reaction scheme may be written:

Reaction	Rate	
$2ROOH \rightarrow RO\cdot + ROO\cdot + H_2O$	$k_i[ROOH]^2$	(25)
$RO\cdot + R{:}H \rightarrow ROH + R\cdot$	$k_r[RO\cdot][R{:}H]$	(26)
$R\cdot + O_2 \rightarrow ROO\cdot$	Fast	(27)
$ROO\cdot + R{:}H \rightarrow ROOH + R\cdot$	$k_p[ROO\cdot][R{:}H]$	(28)
$2ROO\cdot \rightarrow$ Inactive products	$k_t[ROO\cdot]^2$	(29).

It is assumed again that the concentration of free radicals in the system reaches a constant value and that a negligible amount of R:H is consumed by the reaction shown in Equation (26) compared to that shown in Equation (28). Proceeding as before, the rate of free-radical production may be equated to the rate of free-radical removal:

$$k_i[ROOH]^2 = k_t[ROO\cdot]^2 \qquad (30).$$

Solving for $[ROO\cdot]$:

$$[ROO\cdot] = (k_i/k_t)^{0.5}[ROOH] \qquad (31).$$

The rate of reaction of R:H is:

$$-d[R{:}H]/dt = k_p[ROO\cdot][R{:}H] \qquad (32).$$

By substitution for $[ROO\cdot]$, Equation (32) becomes:

$$-d[R{:}H]/dt = k_p(k_i/k_t)^{0.5}[ROOH][R{:}H] \qquad (33).$$

In an autocatalytic oxidation, the rate of reaction is proportional to the concentrations of the hydroperoxide and the organic compound.

PRESENTATION OF RELATED STUDIES

The following discussion illustrates that organic compounds have been observed experimentally to react with molecular oxygen according to the oxygen-initiated kinetic scheme previously developed. According to this scheme, reaction

orders of 1.5 with respect to the concentration of the organic compound and 0.5 with respect to the concentration of oxygen would be expected.

Bolland and Gee (39) studied the uncatalyzed oxidation of ethyl linoleate and concluded that the reaction proceeded by the direct interaction of molecular oxygen with the hydrocarbon. The rate of reaction was found to be proportional to the 1.5 and 0.5 powers of the ethyl linoleate concentration and oxygen pressure, respectively. The activation energy determined experimentally was approximately 26 kcal./mole.

Russell (51) investigated the liquid-phase oxidation of indene, an unsaturated compound, in the presence and absence of a free-radical generator at 50°C. and atmospheric pressure. In the absence of the initiator, he found that the reaction rate was proportional to the 1.5 power of the indene concentration and to the 0.5 power of the oxygen concentration. He concluded that the initial reaction was the direct interaction of oxygen with indene.

A study of the oxidation of styrene in solution over the temperature range from 35 to 85°C. and with oxygen pressures from 0.1 to 4.0 atmospheres was conducted by Miller and Mayo (52). In the absence of an initiator, they determined that the rate of reaction was of order 1.4 and 0.4 with respect to the styrene and oxygen concentrations, respectively, in good agreement with theory. They concluded that oxygen participated directly in the initiation reaction. In the temperature range studied, the activation energy was 23.0 kcal./mole.

After investigating the kinetics of the oxidation of n-decanal, Cooper and Melville (40) deduced both on empirical and theoretical grounds that the reaction rate was proportional to the 1.5 power of the n-decanal concentration and the 0.5 power of the oxygen concentration. They concluded that oxygen initiated the reaction directly at the aldehydic carbon atom.

The oxidation of ground ethyl cellulose film (approximate degree of substitution 2.5) under one atmosphere of oxygen in the temperature interval from 69 to 108°C. has been studied by McBurney (50). The activation energy of the reaction was found to be 25 kcal./mole. Koz'mina and Kurlyankima (53) investigated the same reaction over the temperature range from 100 to 130°C. From the rate of oxygen absorption, the overall energy of activation was calculated to be 26.7 kcal./mole, while a value of 27.4 kcal./mole was calculated from the length of the induction period at the various temperatures studied.

The oxidation of methyl glycopyranosides, principally methyl β-D-glucopyranoside as a cellulosic model compound, was studied by Church (24). The homogeneous oxidation of molten methyl β-D-glucopyranoside under 17.2 atmospheres of oxygen in the temperature range from 108 to 130°C. was found to be of order 1.5 with respect to the concentration of glucopyranoside. The activation energy was found to be 26.4 kcal./mole. He concluded that the reaction proceeded by a free-radical mechanism initiated directly by molecular oxygen.

THE OXIDATION OF GLUCOSE IN AQUEOUS SOLUTION

Although molecular oxygen is known to oxidize a variety of organic compounds, the oxidation of simple carbohydrates in aqueous solution by oxygen has not been investigated previously. The present work, in which the oxidation of D-glucose in solution was studied, was undertaken to provide information regarding the nature of the reaction products and the manner in which they are formed. The results of this work are discussed in this and the following sections.

In the preceding sections, it was shown that the attack of molecular oxygen on organic compounds containing labile hydrogen atoms proceeds by free-radical chain processes. The position of attack is preferentially at points of high electron density or where the resultant radical may be stabilized by resonance. Furthermore, it was shown that under certain conditions the diradical oxygen molecule may initiate the reaction directly.

In this work the oxidation by molecular oxygen of glucose in aqueous solution was studied over the temperature interval from 110 to 140 °C. The reactions were conducted in a high-pressure apparatus fitted with a Teflon insert, and solutions of highly purified glucose in triply-distilled water were used to avoid metal ion catalysis. Generally, the product analysis work was performed by the technique of isotope dilution. (The descriptions of the oxidation apparatus, the reagents, and the general oxidation and analytical procedures are presented in the Experimental section, p. 63.) That glucose does react under these conditions is shown by the data in Table I. The nature of the products indicates that glucose was degraded by a series of oxidative reactions.

It is well known that free-radical reactions are susceptible to inhibition by certain types of compounds. These compounds, termed antioxidants, are able

TABLE I

THE OXIDATION OF GLUCOSE IN AQUEOUS SOLUTION BY MOLECULAR OXYGEN[a] AT 140°C.

			Analyses of Solutions After Reaction						
Oxidation Number	Time, hr.	Initial Glucose Concn.,[b]	Unreacted Glucose[b]	Specific Rotation, degrees[c]	pH[d]	Gluconic Acid[b]	Arabinose[b]	Arabonic Acid[b]	Carbon Dioxide, mg.
39	4	5.0000	4.3565	+49.2	2.92	0.0551	0.0133	0.0128	--
40	8	5.0000	3.8097	+48.1	2.61	0.1170	0.0169	0.0172	--
41	12	5.0000	3.2248	+44.9	2.40	0.2256	0.0221	0.0185	--
42	16	5.0000	2.3598	+36.9	2.17	0.3999	0.0270	0.0373	--
27	20	5.0000	1.5635	+24.9	2.11	0.4030	0.0168	0.0639	215.1

[a]The initial oxygen pressure was 50 atm. at 25°C.

[b]The concentration is expressed as g./100 ml. of solution.

[c]The initial equilibrium specific rotation, $[\alpha]_D$, of glucose in aqueous solution is +52.7°.

[d]The pH of the solutions before reaction was 6.65.

to terminate the reaction chains without starting new chains. The effect of hydroquinone, a frequently used antioxidant (25), on the oxidation of glucose was examined. Generally, the reaction was followed by determining the amount of unreacted glucose at various times at each temperature. However, because of difficulties to be discussed shortly hereafter, it is more revealing to present the results in terms of the effect of hydroquinone on the production of gluconic acid (the primary product) rather than on the amount of unreacted glucose. The inhibitory effect noted is strong evidence that a free-radical mechanism was operative during the oxidation (Table II).

TABLE II

INHIBITORY EFFECT OF HYDROQUINONE ON THE OXIDATION OF GLUCOSE

Oxidation Number	Glucose[a]	Hydroquinone[a]	Temp., °C.	Time, hr.	Gluconic Acid Formed[a]
41	5.0000	0.0000	140	12	0.2256
46	5.0000	0.5000	140	12	0.0750[b]

[a]The concentration is expressed as g./100 ml. of solution.

[b]Determined by paper chromatography.

KINETICS OF THE OXIDATION OF GLUCOSE IN AQUEOUS SOLUTION

The kinetic data in this study were obtained at reaction temperatures of 110, 125, and 140°C. At each temperature, the amount of unreacted glucose at various time intervals was determined by isotope dilution. The data obtained in this manner are tabulated in Table III.

OXYGEN-INITIATED KINETICS

Previously, a rate expression was derived for the rate of consumption of an organic compound during a free-radical oxidation initiated directly by molecular

TABLE III

KINETIC DATA FOR THE OXIDATION OF GLUCOSE

Oxidation Number	Time, hr.	Initial Glucose, g./100 ml.	Unreacted Glucose, g./100 ml.	Reaction of Glucose, %
Temperature: 110°C.				
45	24	5.0000	4.8757	2.5
33	48	5.0000	4.4949	10.1
35	72	5.0000	4.1837	16.4
36	96	5.0000	3.8891	22.2
38	192	5.0000	2.8303	43.4
Temperature: 125°C.				
31	12	5.0000	4.3924	12.2
32	18	5.0000	4.0297	19.4
28	24	5.0000	3.8059	23.9
29	48	5.0000	2.7612	44.8
30	72	5.0000	1.5366	69.2
Temperature: 140°C.				
39	4	5.0000	4.3565	12.8
40	8	5.0000	3.8097	23.8
41	12	5.0000	3.2248	35.6
42	16	5.0000	2.3598	52.8
27	20	5.0000	1.5635	68.8

oxygen. The equation was (p. 15):

$$-d[R:H]/dt = k_p(k_i/k_t)^{0.5}[R:H]^{1.5}[O_2]^{0.5} \qquad (24).$$

Combination of the three individual rate constants into one overall constant, $\underline{k}_a$, yields:

$$-d[R:H]/dt = k_a[R:H]^{1.5}[O_2]^{0.5} \qquad (34).$$

The concentration of oxygen in the liquid phase, $[O_2]$, is assumed to follow Henry's Law:

$$[O_2] = k_h P_{O_2} \qquad (35)$$

where $\underline{k}_h$ is Henry's Law constant. At constant temperature in the presence of a large excess of oxygen in the gas phase, the concentration of oxygen in the liquid phase will remain approximately constant if it is assumed that $\underline{k}_h$ does not change appreciably upon reaction of R:H with oxygen. Since the concentration of oxygen in the liquid phase is raised to the 0.5 power, small changes in its value will not greatly affect the overall rate of reaction. Substituting the symbol $\underline{k}_o$ for $[O_2]^{0.5}$, the rate equation is now:

$$-d[R:H]/dt = k_a k_o [R:H]^{1.5} \qquad (36).$$

Integration of Equation (36) yields the following:

$$2(1/[R:H]^{0.5} - 1/[R:H]_o^{0.5}) = k_a k_o t \qquad (37)$$

where $[R:H]_o = [R:H]$ at $\underline{t} = 0$. It is seen from Equation (37) that a plot of the left side of the equation versus time (at constant temperature) should result in a straight line. Agreement of the experimental data with theory would indicate that the oxidation of the compound under study is initiated directly by molecular oxygen.

The data of Table III have been calculated in accordance with Equation (37) and are presented in Table IV and Fig. 1. From Fig. 1 it may be seen that the data are consistent with the oxygen-initiated kinetic scheme up to approximately 35-40% reaction, which corresponds to the linear portions of the curves. At higher degrees of reaction (i.e., at longer reaction times), the manner of deviation of the data indicates that more glucose has reacted than would be expected from the straight-line relationship observed below approximately 35-40% reaction. The reason for this deviation will be discussed in a succeeding section.

TABLE IV

KINETIC DATA FOR OXYGEN-INITIATED REACTION

Oxidation Number	Temperature, °C.	Time, hr.	$2(1/[R:H]^{0.5} - 1/[R:H]_o^{0.5})$
45	110	24	0.0464
33	110	48	0.2075
35	110	72	0.3539
36	110	96	0.5082
38	110	192	1.2495
31	125	12	0.2541
32	125	18	0.4324
28	125	24	0.5550
29	125	48	1.3123
30	125	72	3.0519
39	140	4	0.2707
40	140	8	0.5528
41	140	12	0.9308
42	140	16	1.7298
27	140	20	3.0101

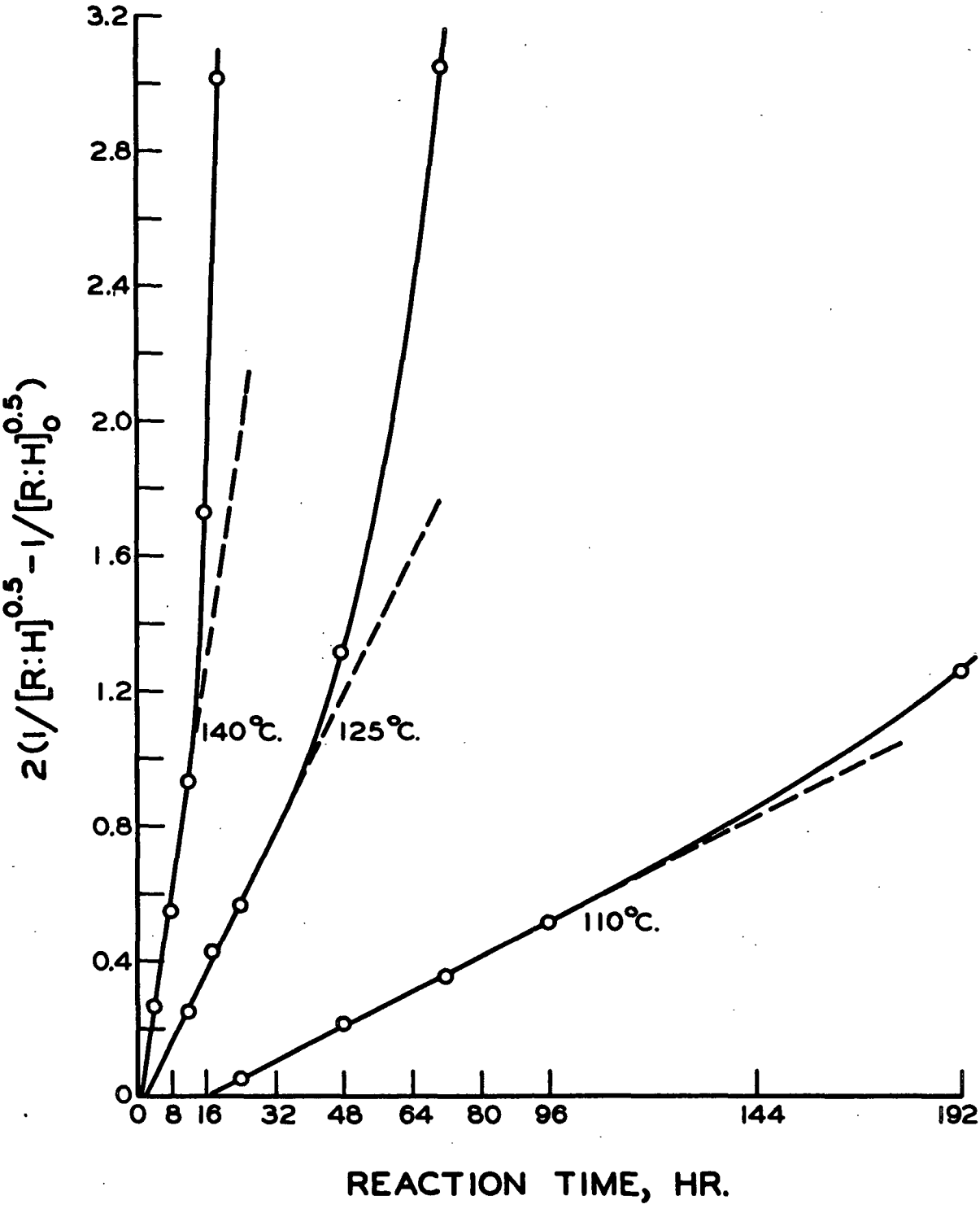


Figure 1. Oxygen-Initiated Kinetic Data for Glucose

AUTOCATALYTIC KINETICS

Some reactions in which molecular oxygen is the oxidant and which proceed by a free-radical chain mechanism are observed to be autocatalytic in nature. This phenomenon frequently occurs in olefinic oxidations and results from the decomposition of the hydroperoxides into radical products. Although there is evidence for the presence of peroxide compounds among the oxidation products of glucose, autocatalysis was not observed. Cooper and Melville (40) and Church (24) did not observe the phenomenon of autocatalysis in the oxidation of n-decanal or methyl β-D-glucopyranoside, respectively. The oxidation of these compounds, both of which have structural features similar to glucose, was shown to be initiated directly by molecular oxygen.

The rate expression previously derived (p. 16) for an autocatalytic reaction was:

$$-d[R:H]/dt = k_p(k_i/k_t)^{0.5}[ROOH][R:H] \qquad (33).$$

Combining the individual rate constants into one constant, k_a, Equation (33) becomes:

$$-d[R:H]/dt = k_a[ROOH][R:H] \qquad (38).$$

If ROOH is the initial product of the reaction (which is true in many olefinic oxidations), its concentration is given by:

$$[ROOH] = [ROOH]_o + [R:H]_o - [R:H] \qquad (39)$$

where $[ROOH]_o$ and $[R:H]_o$ are the initial concentrations of ROOH and R:H, respectively. By substitution the complete rate equation is:

$$-d[R:H]/dt = k_a[R:H]([ROOH]_o + [R:H]_o - [R:H]) \qquad (40).$$

Equation (40) may be integrated to yield:

$$1/F \ln([ROOH]/[R:H]) = k_a t + 1/F \ln([ROOH]_o/[R:H]_o) \qquad (41)$$

where $\underline{F} = [ROOH]_o + [R:H]_o$. If $[ROOH]_o$ is assumed to be small, then [ROOH] is approximately the amount of R:H that has reacted. For an autocatalytic reaction, a plot of $\ln([ROOH]/[R:H])$ versus time, $\underline{t}$, should be linear with slope $\underline{Fk_a}$ and $\underline{y}$-intercept $\ln([ROOH]_o/[R:H]_o)$. The data from Table III for glucose have been calculated in accordance with Equation (41) and are presented graphically in Fig. 2. It may be seen that the deviation of the data from linearity is great, particularly at the lower temperatures, and that the oxidation of glucose by molecular oxygen is not well described by an autocatalytic reaction scheme. The apparent agreement of the data with theory at 140°C. may be due, in part, to a slight autocatalytic effect. However, it is felt that the primary reason for the accord is the increased amount of 5-hydroxymethylfurfural formed from glucose at this temperature. The latter subject is discussed in the following section.

DEVIATION OF KINETIC DATA

The graphical presentation of the kinetic data in Fig. 1 (p. 24) shows that more glucose has reacted at the longer reaction times at each temperature than would be predicted from the straight-line relationship observed at the lower reaction times. In addition, a progressive darkening of the solutions from colorless through yellow to amber with time as well as the formation of a water-insoluble, dark brown material at the longer times was observed in this work. It is postulated that this behavior is due to the formation of 5-hydroxymethyl-furfural (HMF) from glucose which subsequently polymerizes to give the solid, brown material noted. Arabinose, a reaction product, may also contribute to the darkening of the solutions with time since it is the precursor of furfural.

Furfural and HMF are normally prepared by the action of hot strong acids on pentoses and hexoses, respectively (54). However, Wolfrom, et al. (55) have

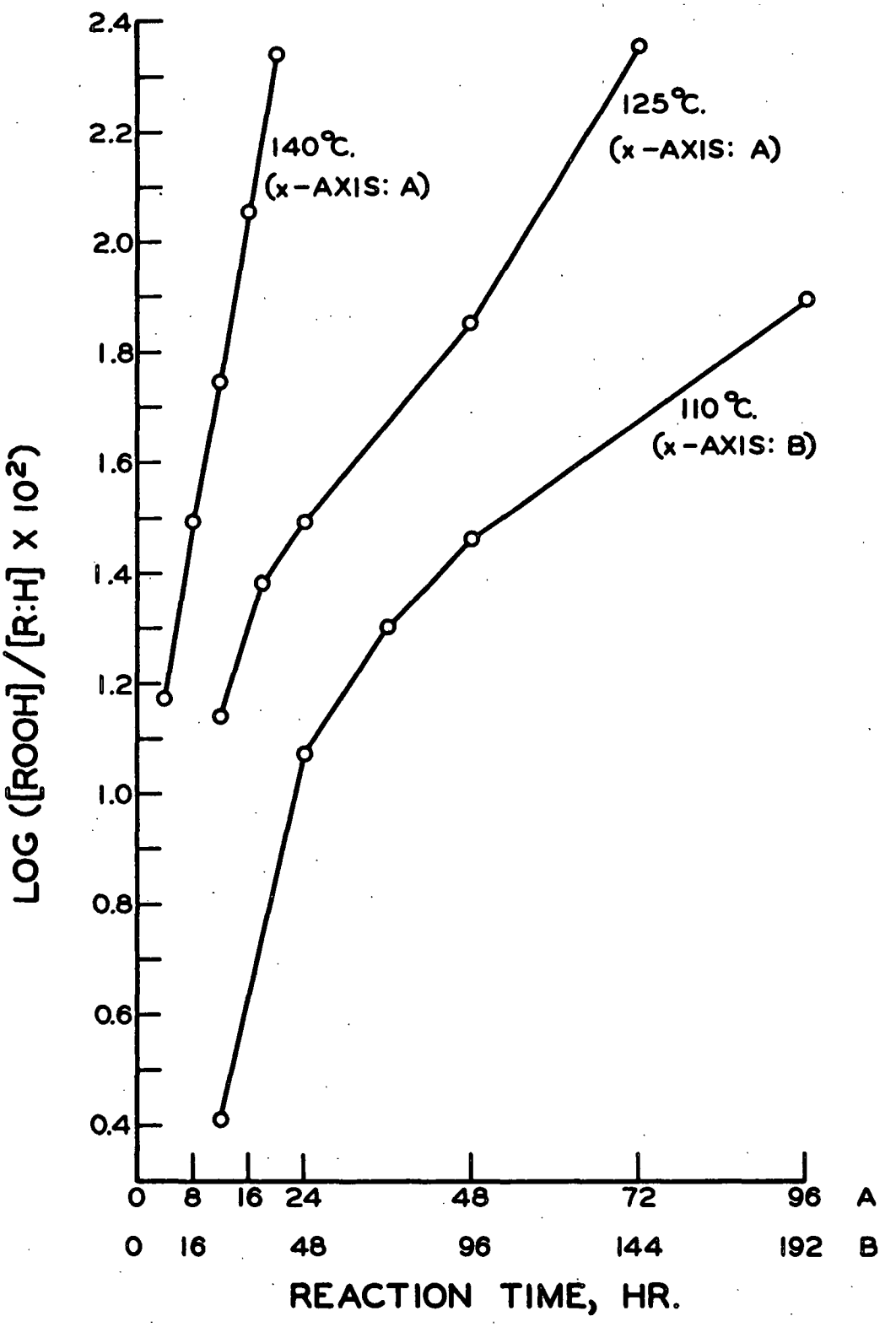


Figure 2. Nonconformity of Glucose Oxidation with Autocatalytic Scheme

reported the formation of HMF in aqueous glucose solutions heated at 100-150°C. It has also been shown (56) that HMF (which results from the degradation of glucose) is the major precursor of the discoloring materials found in starch hydrolyzates.

It appears from the literature (57-61) that furfural (and presumably HMF) is degraded by autoxidative free-radical processes or by ionic processes under acidic conditions in aqueous solution to a variety of complex polymeric or resinous materials. The similarity of the conditions necessary for the formation and degradation of HMF and furfural to those used in the present work has led to the conclusion that reactions of this nature account for the development of color and the formation of solid material in the reaction solutions. These reactions are believed to account for the deviation of the data noted at the longer reaction times. Analogous reactions are thought to occur in the oxidation of molten methyl β-D-glucopyranoside (24). At 85% reaction of the glucoside, the polymeric material was estimated to constitute approximately 45% of the total products.

Furfural and HMF show absorption maxima in the ultraviolet region of the spectrum at 277.5 and 284 millimicrons, respectively (62). Ultraviolet spectra were determined for all of the reaction solutions. The absorption maximum occurs near 284 millimicrons initially and then decreases towards 277.5 millimicrons as the reaction time increases. This behavior is to be expected as the amount of arabinose, and, hence, furfural present increases. The data are presented in Table V. In the calculation it was assumed that HMF was the only absorbing species present. It should be noted that the calculated amount of HMF is probably low, particularly at the longer reaction times, since it does not include that which may have polymerized to yield in part, the insoluble material at the longer reaction times at each temperature.

TABLE V

FORMATION OF 5-HYDROXYMETHYLFURFURAL FROM AQUEOUS GLUCOSE SOLUTIONS

Oxidation Number	Time, hr.	Wavelength of Absorbance Maximum, nm.	Concentration of HMF, mM/100 ml.	Glucose Converted, %
Temperature: 110°C.				
45	24	282.5	0.044	0.16
33	48	281.0	0.099	0.36
35	72	280.0	0.119	0.43
36	96	279.0	0.129	0.46
38[a]	192	278.0	0.082	0.30
Temperature: 125°C.				
31	12	282.0	0.083	0.30
32	18	281.5	0.132	0.48
28	24	278.5	0.192	0.69
29[a]	48	274.5	0.191	0.69
30[a,b]	72	--	--	--
Temperature: 140°C.				
39	4	283.0	0.195	0.70
40	8	282.5	0.281	1.01
41	12	280.0	0.274	0.99
42[a]	16	270.0	0.320	1.15
27[a,c]	20	--	--	--
46[a,d]	12	280.0	0.699	2.52

[a] Water-insoluble material present.

[b] No definite maximum in the absorption curve.

[c] Spectrum not obtained due to consumption of solution in other analyses.

[d] Reaction in which hydroquinone was added to the glucose solution.

Generally, the data in Table V show that the amount of HMF increases during the early stages of the reaction and then tends to level off at the longer reaction times. The latter behavior corresponds roughly to the appearance of the insoluble material. The amount of HMF formed in Oxidation 46, in which hydroquinone was

added to the solution, is about two and one-half times that formed in Oxidation 41. Additionally, solid material was present also. It was for this reason that the effect of hydroquinone as an antioxidant was evaluated with respect to the formation of gluconic acid rather than with respect to the consumption of glucose.

In order to verify the presence of HMF, an aliquot of Solution 28 (24 hours at 125°C.) was taken and the 2,4-dinitrophenylhydrazone prepared according to the procedure of Blanksma and Wackers (63). The melting point of the product was 183-185°C.; literature value: 184°C.

DETERMINATION OF THE ENERGY OF ACTIVATION

In free-radical reactions it is usually assumed that each of the individual rate constants for the initiation, propagation, and termination reactions has an Arrhenius temperature dependence. With this assumption it may be shown (64) that the overall rate constant, k_a, will also have an Arrhenius temperature dependence according to Equation (42):

$$d(\ln k_a)/dT = E_a/RT^2 \qquad (42)$$

where T = absolute temperature,

R = gas constant,

E_a = overall activation energy.

The integration of the latter equation by indefinite integrals yields:

$$\log k_a = -E_a/2.303RT + C \qquad (43)$$

where C = constant of integration. The energy of activation may be evaluated from the slope of the plot of $\log k_a$ versus $1/T$.

It was shown previously that the oxidation of glucose in aqueous solution by molecular oxygen is well described up to approximately 35-40% reaction by the

oxygen-initiated kinetic scheme. Values of $\underline{k}_a\underline{k}_o$ at each temperature may be determined from Equation (37) or from the slopes of the straight lines in Fig. 1. If $\underline{k}_o$ were temperature-independent, then the values of $\underline{k}_a\underline{k}_o$ could be used in place of $\underline{k}_a$ to determine the energy of activation.

It may be seen from Equation (35) that:

$$k_o = (k_h P_{O_2})^{0.5} \qquad (44).$$

Generally, the solubility of a gas in a liquid decreases as the temperature is raised due to decreases in $\underline{k}_h$. In this work, however, a sealed bomb charged to 50 atm. at 25°C. was used in each reaction. Therefore, as the temperature of the bomb is raised, the internal pressure will increase. With increasing temperature the two opposing processes tend to offset one another and the product $\underline{k}_h\underline{P}_{O_2}$ will remain reasonably constant. Additionally, the dependence of $\underline{k}_o$ on the 0.5 power of the product $\underline{k}_h\underline{P}_{O_2}$ helps to further minimize the effect of small variations in the latter on the overall reaction rate. Church (24) has shown that $\underline{k}_o$ varies by only 1% over the temperature range from 80 to 100°C. with water in a similar system. It will be assumed that $\underline{k}_o$ remains essentially constant over the temperature range in question, and that values of $\underline{k}_a\underline{k}_o$ may be used in place of $\underline{k}_a$ to calculate the energy of activation. The values of $\underline{k}_a\underline{k}_o$, obtained from Fig. 1 by linear multiple regression analysis, are tabulated in Table VI.

In Fig. 3 the logarithms of the rate constants are plotted versus the reciprocal of the absolute temperature. The straight-line relationship predicted from the Arrhenius equation is obtained. From the slope of the line (determined by linear multiple regression analysis), the energy of activation was calculated to be 26.8 kcal./mole. This value compares favorably with the energies of activation determined by others for oxidative reactions initiated directly by molecular oxygen. These studies were presented earlier on p. 16.

TABLE VI

RATE CONSTANTS FOR OXYGEN-INITIATED OXIDATION OF GLUCOSE

Temperature, °C.	Rate Constant, $k_a k_o \times 10^4$, $(mole/l.)^{-1/2} hr.^{-1}$	Log ($k_a k_o \times 10^4$)
110	63.8	1.8048
125	250.7	2.3991
140	825.1	2.9166

PRODUCTS OF THE OXIDATION OF GLUCOSE IN AQUEOUS SOLUTION

QUALITATIVE CHROMATOGRAPHY

In the early phases of the product analysis work, descending paper chromatography was used extensively to determine the nature of the reaction products in solution. The major components were identified by comparing their behavior with that of known compounds in various developers and with several spray reagents. The chromatographic techniques, developers, and spray reagents are described in Appendix IV.

The products identified during this work were gluconic acid, arabinose, arabonic acid, and erythronic acid. In addition, erythrose and 2-ketogluconic acid were tentatively identified in several developers with various spray reagents. Generally, however, it was difficult to separate these compounds completely from other components of the reaction solutions. Some streaking of the chromatograms run in acidic developers was apparent in the disaccharide region. Since no discrete spots were observed and the materials were present in very small amounts, they were not investigated further. Notably neither glucuronic acid nor xylose were detected in the reaction products indicating that oxidation did not occur at the C6 position. Church (24) did not detect glucuronic acid among the products from the oxidation of crystalline methyl β-D-glucopyranoside.

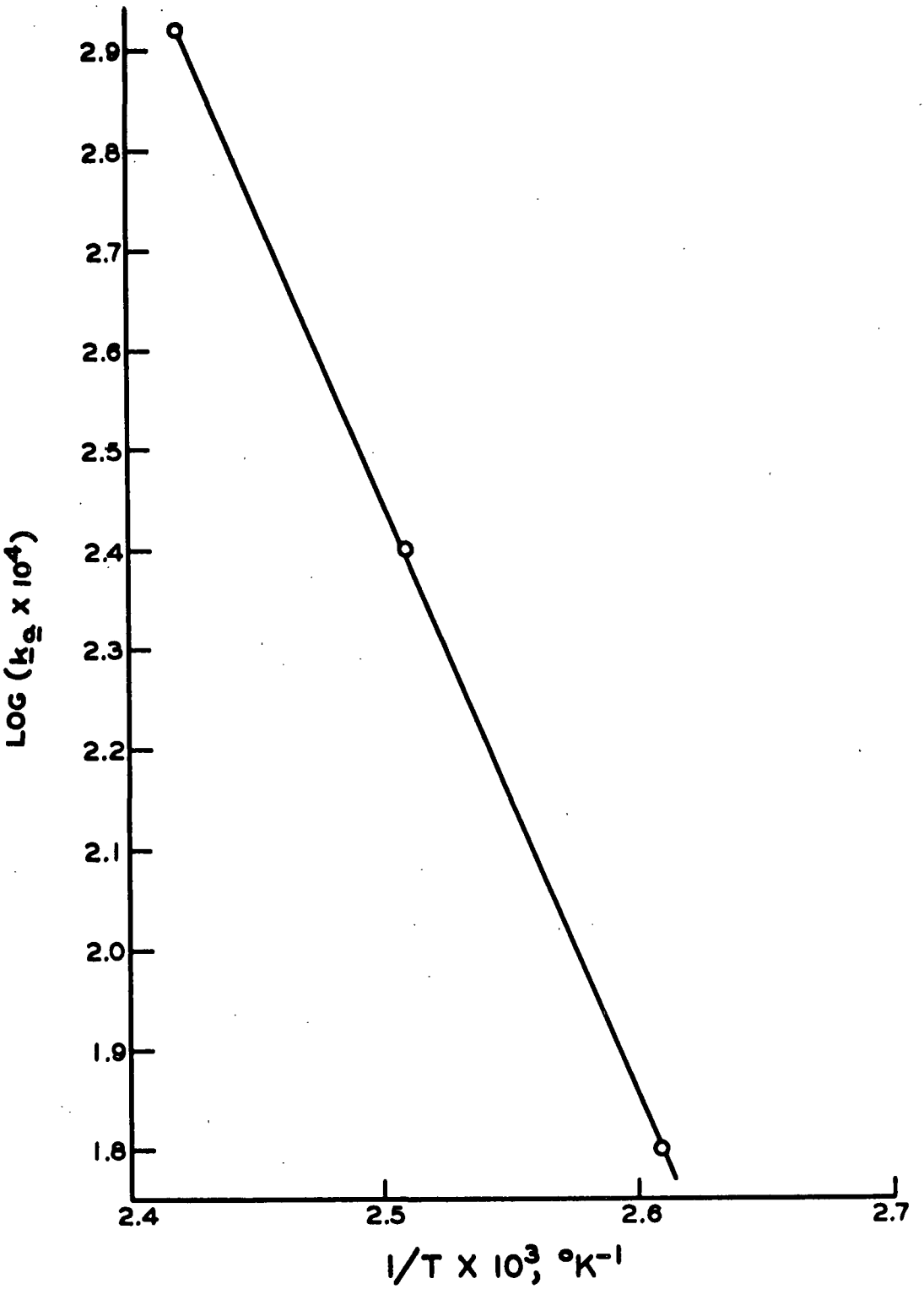


Figure 3. Arrhenius Diagram for Glucose Oxidation

A few qualitative observations pertinent to the general course of the reaction can be made from examining the chromatograms prepared from successive reaction solutions at each temperature. Gluconic acid is the initial major reaction product. Comparatively small amounts of the other products are present in solutions in which the degree of reaction is low. In Solution 45 in which the percentage reaction of glucose was 2.5%, gluconic acid was the only component detected (in addition, of course, to unreacted glucose). Arabinose and arabonic acid appear as reaction products at approximately the same time which is, however, prior to the appearance of erythrose and erythronic acid. Generally, the former pair was detected chromatographically at about 12% reaction of glucose while the latter pair was not detected until approximately 24% reaction. These observations are suggestive of an oxidative sequence in which glucose is degraded in a one-carbon, stepwise manner.

ISOTOPE DILUTION DATA

The principal analytical technique used for the quantitative determination of the major oxidation products revealed by chromatographic investigation was isotope dilution. The technique, as employed in the present work, involved the addition of the inactive (i.e. unlabeled) form of the desired constituent to the reaction mixture. The constituent was then isolated by suitable means and its reduced specific activity measured. From the original specific activity of the starting material and the amount of inactive constituent added, the amount of product formed during the reaction was calculated. A complete description of the technique and the manner of its application is presented in Appendix I.

Gluconic Acid, Arabinose, and Arabonic Acid

The formation of gluconic acid, arabinose, and arabonic acid with time at 140°C. was studied with the reaction solutions used to obtain the kinetic data.

Gluconic acid was isolated as its phenylhydrazide derivative from an aliquot of each solution. Arabinose and arabonic acid were determined from a second aliquot of each solution. They were isolated as their diphenylhydrazone and phenylhydrazide derivatives, respectively, after separation of the aliquot into a neutral and an acidic fraction with ion-exchange resins. The results of this work are presented in Table VII and Fig. 4. The reactions leading to the formation of these products will be discussed during the presentation of the oxidation sequence.

TABLE VII

FORMATION OF GLUCONIC ACID, ARABINOSE, AND ARABONIC ACID FROM GLUCOSE[a] AT 140°C.

Oxidation Number	Time, hr.	Unreacted Glucose, mM/100 ml.	Gluconic Acid, mM/100 ml.	Arabinose, mM/100 ml.	Arabonic Acid, mM/100 ml.
39	4	24.20	0.28	0.09	0.08
40	8	21.17	0.60	0.11	0.10
41	12	17.92	1.15	0.15	0.11
42	16	13.11	2.04	0.18	0.22
27	20	8.64	2.06	0.11	0.38

[a]The initial concentration of glucose in each solution was 27.78 mM/100 ml.

The experimental data shown in Fig. 4 indicate that gluconic acid is produced more rapidly than arabinose or arabonic acid during the oxidation of glucose. This observation confirms the tentative conclusion drawn from the chromatographic data that gluconic acid is the initial reaction product.

The formation of gluconic acid as the primary product of the reaction is in accord with the earlier theoretical considerations regarding the position of attack, and indicates that the initial attack is at C1. The anomeric carbon atom

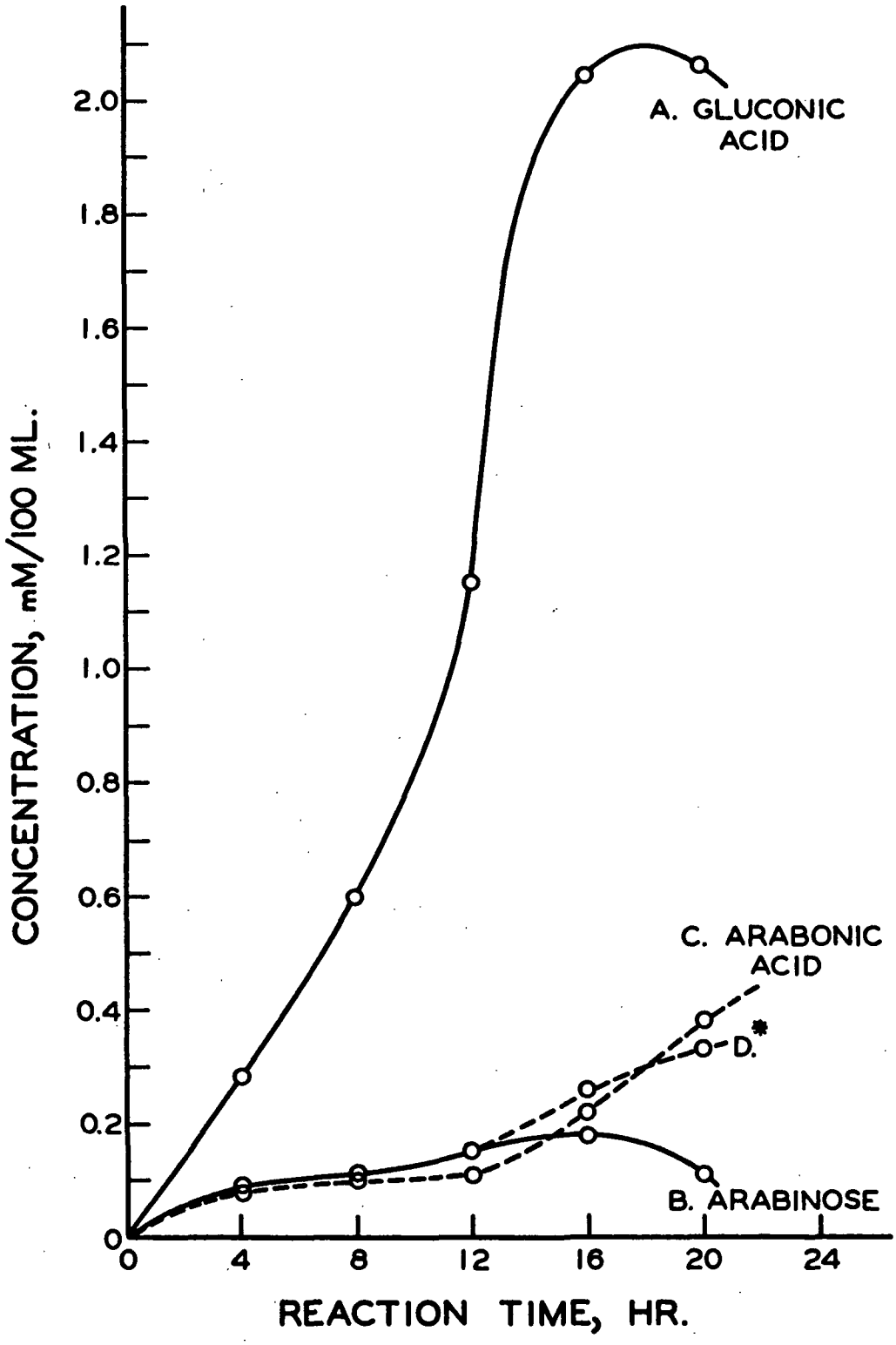


Figure 4. Formation of Gluconic Acid, Arabinose, and Arabonic Acid with Time at 140°C.

* See p. 39.

is bonded to two oxygen atoms compared to the other carbon atoms which are only bonded to one oxygen atom. The possibilities for resonance stabilization of a radical formed by removal of a hydrogen atom are greater in the former situation, and it would be expected that attack would occur principally at this point. Additional experimental evidence is presented in the following section which further substantiates this conclusion.

Arabinose and arabonic acid are secondary reaction products formed from gluconic acid. During the initial stages of the reaction, it appears from Fig. 4 that they are formed at approximately the same rate. It is expected a priori that arabonic acid would be produced from arabinose in the same manner as gluconic acid is produced from glucose. Therefore, it is unlikely that arabonic acid is formed exclusively from gluconic acid at the same rate that arabinose is formed. Alternatively, if arabonic acid were formed solely from arabinose, its concentration would not parallel that of arabinose. (The relative manner in which the concentrations of arabinose and arabonic acid varied with time would depend on the magnitude of the rate constants for their respective formations.) It is concluded that arabonic acid is formed from both arabinose and gluconic acid. However, the data are not sufficient to draw any firm conclusions with respect to the relative rates of these reactions.

In the period from zero to twelve hours, it is seen from Fig. 4 that the rate of formation of gluconic acid increases continuously, particularly during the period from four to twelve hours. The rate of formation of arabinose also increases similarly during the latter period. This type of behavior is suggestive of an autocatalytic reaction. Some autocatalysis may occur. However, since it was shown earlier that the kinetic data are not, in general, well described by an autocatalytic reaction scheme, another factor may be of importance in accounting for these observations. The increasing rates of formation of gluconic acid and

arabinose may be explained by considering the changes in pH within the system during the reaction.

In the system under consideration, there are two competing reaction schemes, both of which consume glucose and arabinose. These reactions involve the oxidation of glucose and arabinose by molecular oxygen, and the dehydration of these compounds to HMF and furfural, respectively.

Singh, et al. (56) have studied the formation of HMF from glucose as a function of the initial pH of the solution. Dilute hydrochloric acid was used to adjust the pH to its initial value. Their results demonstrated that glucose is remarkably stable with respect to the formation of HMF in the region of pH 3, due to the stability of the ring structure of glucose at this pH. Their data for a ten percent glucose solution which had been heated at 145°C. for one-half hour are reproduced below. The minimum noted with glucose was not observed with fructose or sucrose.

The work of Singh, et al. shows that the rate of formation of HMF decreases with decreasing pH and reaches a minimum value in the region of pH 3.5 to 2.5. During the oxidation of glucose, the pH of the solution decreases continually from an initial value of 6.65 through values of 2.92, 2.61, 2.40, 2.17, and 2.11 at reaction times of four, eight, twelve, sixteen, and twenty hours, respectively. Thus, the rate of formation of gluconic acid would be expected to increase as the rate of formation of HMF decreases. The period from four to twelve hours in which the most rapid increase in the rate of gluconic acid formation is noted corresponds to the region of pH in which the minimum with respect to the rate of HMF formation is observed. Since arabinose is produced from gluconic acid, an increase in the rate of formation of the latter would be expected to yield an

increase in the rate of formation of the former. This behavior is apparent from Fig. 4 in the region from four to twelve hours.

The observed decrease in the rate of formation of gluconic acid at reaction times greater than twelve hours is again attributable, in part, to the effect of pH (as predicted from Fig. 5) on the rate of HMF formation.

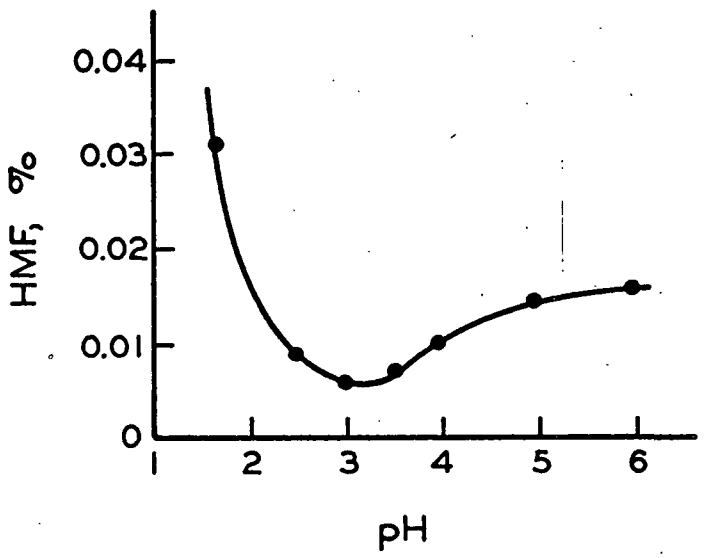


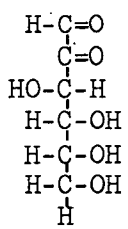
Figure 5. Formation of HMF versus pH (56)

The formation of furfural from xylose at 160°C. has been studied by Dunlop (59). In contrast to the results of Singh, et al. obtained for glucose, Dunlop found that the rate of furfural formation is proportional to the concentration of xylose at constant pH and to the hydrogen ion concentration. In addition, the rate was observed to approximately double for each 10°C. increase in temperature. The data of Dunlop were used to estimate the effect of this reaction on the amount of arabinose determined at the various reaction times in the present work, since no data were found pertaining to the rate of formation of furfural from arabinose.

The data are presented in Table VIII in terms of the amount of furfural formed during each four-hour time interval. The mean pH during each four-hour period was used to calculate the rate constant for that period. It is seen from Table VIII that the effect of pH upon the rate of furfural formation is very pronounced at the longer reaction times. During the periods from twelve to sixteen hours and from sixteen to twenty hours, approximately one half of the arabinose produced from gluconic acid is converted into furfural. In the absence of this competing reaction, the maximum concentration of arabinose would be higher than that shown in Fig. 4 and would not be reached until somewhat after twenty hours. This is illustrated in Fig. 4 by the dotted line D.

Glucosone

Several of the reaction solutions used in the kinetic work were examined for the presence of glucosone (D-arabinohexosone) in order to determine if this compound was an important intermediate product from the reaction. This was accomplished



D-Glucosone

by comparing the amount of unreacted glucose determined as β-D-glucose pentaacetate (GPA) with the amount of unreacted glucose determined as D-glucose phenylosazone (GØØ). (The measurements were made by the technique of isotope dilution.) If glucosone were present, the amount of unreacted glucose determined as GØO should exceed that determined as GPA since glucosone contributes to the former and not to the latter quantity. From the results reported in Table IX, it was concluded that glucosone is not an important intermediate product in the

TABLE VIII

ESTIMATED FURFURAL FORMATION FROM ARABINOSE AT 140°C.

Oxidn. No.	Time, hr.	pH	Arabinose Determined, mM/100 ml.	Calculated Furfural Formed in 4-hr. Period, mM/100 ml.	Percent Conversion of Arabinose in 4-hr. Period, %	Total Calculated Furfural, mM/100 ml.	Total Arabinose[a] Produced, mM/100 ml.
39	4	2.92	0.09	0.00004	0.1	0.00004	0.09004
40	8	2.61	0.11	0.0018	2.0	0.00184	0.11184
41	12	2.40	0.15	0.0040	3.6	0.0058	0.1558
42	16	2.17	0.18	0.0696	44.7	0.0754	0.2554
27	20	2.11	0.11	0.1447	56.6	0.2201	0.3301

[a]Total arabinose includes the amount of arabinose determined experimentally plus that calculated as being converted into furfural.

present study. These data support further the conclusion from the previous section that the position of initial attack is at C1.

TABLE IX

EVALUATION OF D-GLUCOSONE AS A REACTION INTERMEDIATE

Oxidn. No.	Temp., °C.	Time, hr.	Unreacted Glucose as GPA, g.	Unreacted Glucose as GØO, g.	Percent Difference[a]
31	125	12	4.3924	4.3413	+1.18
32	125	18	4.0297	4.1043	-1.82

[a]Based on glucose phenylosazone (GØO).

Oxalic Acid

The early appearance of arabinose and arabonic acid as reaction products indicates that the pyranose ring is cleaved between carbon atoms 1 and 2 at low degrees of reaction. If cleavage of the ring also occurred between C2 and C3, two-carbon fragments would be present. From Table VII it is seen that gluconic acid is formed most rapidly in the reaction, and since glucosone is not an intermediate product, it would appear that oxidation at C1 would precede rupture of the C2-C3 bond if rupture occurred at this point. Oxalic acid would be a probable product.

The solutions from Oxidations 31 and 32 were used to confirm the presence of oxalic acid. The data, obtained by isotope dilution, are reported in Table X.

TABLE X

DETERMINATION OF OXALIC ACID

Oxidn. No.	Temp., °C.	Reaction Time, hr.	Oxalic Acid, mg.	Oxalic Acid,[a] %
31	125	12	9.93	0.40
32	125	18	9.41	0.38

[a]Based on the initial amount of glucose, 27.78 mM.

Although the data are not sufficient to justify any firm conclusions (except that oxalic acid is present), it would appear from the essentially constant value obtained that oxalic acid is formed in a somewhat secondary manner apart from the main course of the reaction or that it is subsequently degraded to other products. The former conclusion is supported by the chromatographic data which indicates that the four-carbon products erythrose and erythronic acid do not appear until after the formation of arabinose and arabonic acid. If oxalic acid was a major product, four-carbon compounds would have been detected earlier in the reaction.

VOLATILE AND NONVOLATILE REACTION PRODUCTS

The preceding product analysis work has dealt only with the nature of the reaction products in solution, i.e., the nonvolatile products. From the measurement of the solids content of each solution after reaction, it was concluded that some of the reaction products are volatile compounds.

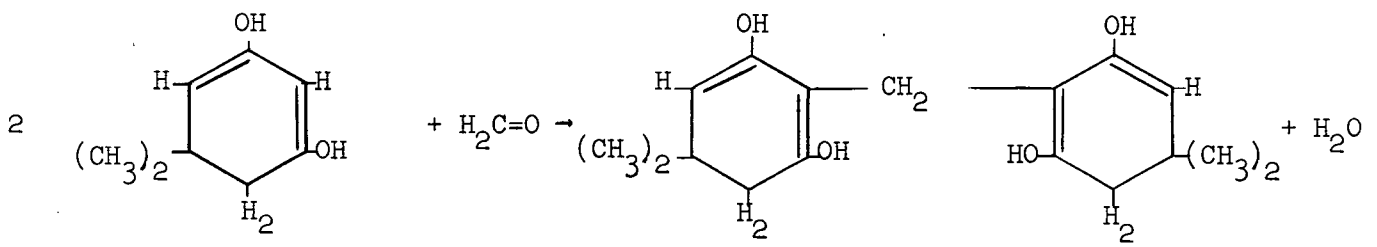
Volatile Products

Church (24) has reported the presence of a volatile acidic compound among the oxidation products of crystalline methyl β-D-glucopyranoside. The gaseous product was determined by absorption in Ascarite-filled tubes but was not identified further. He concluded, however, that it was most probably carbon dioxide. From a survey of recent work concerning the action of gamma-radiation and ultraviolet light during the degradation of carbohydrate materials in aqueous solution [most notably that of Phillips, et al., (11-22)], the most common volatile compounds detected were formaldehyde, formic acid, and carbon dioxide. The presence of these compounds among the oxidation products of the reaction under study was investigated.

Formaldehyde

Pure, dry formaldehyde is a colorless gas which condenses to a liquid at -19°C. and freezes at -118°C. (66). In aqueous solutions, formaldehyde is almost completely hydrated and is present as an equilibrium mixture of the monomeric and polymeric hydrates. In this respect, formaldehyde solutions behave as solutions of a comparatively nonvolatile glycol as opposed to solutions of a volatile gas. The hydrates are relatively stable.

Formaldehyde reacts quantitatively with dimedone, (I), in alkaline, neutral, or mildly acidic aqueous solutions to form a dinuclear methylene derivative called methylene bismethone, (II).



(I) (II) (45)

The product is almost completely insoluble in neutral or acidic aqueous solutions. A sensitivity of approximately 4 p.p.m. is reported with this reagent (66).

In view of the state of formaldehyde in aqueous solution, it was not anticipated that this compound would be detected in the gases relieved from the bomb after an oxidation. This possibility was checked, however, by passing the relief gas from an oxidation slowly through an acidic solution of dimedone in water. (A similar procedure has been used to detect small quantities of formaldehyde in air.) No cloudiness or precipitate formation was noted. However, a precipitate formed immediately upon the addition of a small amount of inactive formaldehyde. The product was examined for radioactivity and found to be inactive. It was concluded that formaldehyde was not present in the relief gas.

The dimedone reagent was also used to examine several of the solutions for the presence of formaldehyde. In conjunction with the attempted identification of formic acid according to the method of Crossman (67), an aliquot from one of the reaction solutions (Solution 27; 20 hours at 140°C.) was taken and diluted with inactive formaldehyde and formic acid. The solution was adjusted to pH 11 and the formaldehyde separated by distillation under reduced pressure. The dimedone derivative was then prepared from the distillate and its specific activity measured. The results, summarized in Table XI, indicate that formaldehyde is not a reaction product. (The counting data are illustrative of those obtained previously from the analysis of the relief gas.)

TABLE XI

OXIDATION 27 – SPECIFIC ACTIVITY OF METHYLENE bis-METHONE

Sample Number	Weight, mg.	Total CPM[a]	Background CPM[a]	Net CPM[a]
1	4.00	152 ± 1	154 ± 1	-2 ± 1
2	4.01	154 ± 1	154 ± 1	0 ± 1

[a] CPM represents the radioactive counts per minute obtained for the sample, the background, and their difference.

Additionally, one solution was examined for the presence of formaldehyde immediately following the relief of the oxidation gases. The dimedone reagent was added directly to an aliquot of the solution. Again, no precipitate was obtained.

Formic Acid

The chromatographic and isotope dilution data both show that the reaction is initiated at C1 with the formation of gluconic acid as the initial product.

In addition, it has also been shown that glucosone and formaldehyde are not reaction products. From these results it is improbable that formic acid is a major product from the oxidation of glucose.

The oxidative cleavage of the bond between carbon atoms one and two in gluconic acid would yield carbon dioxide. The rupture of the C1-C2 bond in glucosone could produce formic acid; however, glucosone is not present. The absence of formaldehyde, a frequent precursor of formic acid in organic oxidation reactions, precludes the formation of the latter in this manner. However, some formic acid may be present, particularly at the longer reaction times, due to the oxidation of HMF and furfural by molecular oxygen. Furfural can be oxidized at room temperature to β-formylacrylic acid and formic acid (58). The former compound polymerizes readily to water-insoluble resinous products. Such products have been noted in this work. Therefore, several of the solutions were investigated for the presence of formic acid.

Formic acid in aqueous solution is not highly volatile due to hydrogen bonding effects. The vapor pressure of the pure liquid at 24°C. is only 40 mm. of mercury, not much greater than that of pure water (68). It was not expected that much formic acid would be lost during the relief of the oxidation gases. Accordingly, the investigations for this compound were confined to the reaction solutions.

Preliminary work with several solid derivatives of formic acid showed that the p-bromophenacyl ester was a suitable derivative for identifying this compound. The derivative was easily prepared according to the method of Hurd and Christ (69). The general procedure was developed by Reid (70), who indicated that derivatization could be accomplished with as little as 100 mg. of material. Solutions with reaction periods of four hours and twenty hours at 140°C. (Solutions 43 and 27, respectively) were examined for formic acid, the former directly and the latter

by the technique of isotope dilution in conjunction with the determination of formaldehyde.

The preparation of the p-bromophenacyl ester of formic acid directly from an aliquot of Solution 43 was unsuccessful. Data obtained by titration indicated that a total of 0.41 meq. of acid was present. Comparing Oxidation 43 with Oxidation 39 in Table VII (p. 35), approximately 0.36 meq. of acid may be attributed to the presence of gluconic and arabonic acids. From Table III (p. 22), it is seen that Oxidation 31 (12 hours at 125°C.) is roughly comparable with Oxidation 39 (4 hours at 140°C.). The former was shown to contain 0.22 meq. of oxalic acid (p. 42). These comparisons show that the total acidity of Oxidation 43 could reasonably be accounted for by gluconic, arabonic, and oxalic acids. If formic acid is present, the amount is very small.

Oxidation 27 was investigated for formic acid by the technique of isotope dilution. After the formaldehyde had been separated from an aliquot of Solution 27 (as discussed in the preceding section), the solution was adjusted to pH 2 and the formic acid distilled under reduced pressure. The aqueous distillate was titrated with standardized sodium hydroxide prior to preparing the p-bromophenacyl ester. From the volume of alkali required, its normality, and the amount of inactive formic acid used for isotope dilution, it was calculated that there was a total of 1.84 meq. of volatile acid in Solution 27. For an unknown reason, the derivative preparation was unsuccessful although formic acid was known to be present, so it was not possible to identify formic acid positively as a reaction product or to check the amount of volatile acid determined by titration by radioactive counting techniques. The acid was assumed to be formic acid and probably arises mainly during the formation of the polymeric material from 5-hydroxymethylfurfural and furfural at the longer reaction times. It was concluded from this

work that formic acid is not a major product from the oxidation of glucose by molecular oxygen.

Carbon Dioxide

Oxidations 44 and 27 (six and twenty hours, respectively, at 140°C.) were investigated to determine if carbon dioxide was a reaction product. After each reaction had been terminated, the gases within the bomb were relieved slowly through a carbon dioxide absorption apparatus consisting of a water vapor trap and two gas absorption bottles connected in series. The absorption bottles contained sodium hydroxide solution to remove carbon dioxide. After the relief gases had been passed through the absorption train, the bomb was flushed with nitrogen until a constant specific activity was obtained from the solution in the first absorption bottle. The amount of carbon dioxide produced during the reaction was calculated from this latter value. During the preliminary work the technique was demonstrated to be accurate to within five percent. (A complete description of the absorption train and the technique is given in the Experimental section, p. 63.) The results of this work are summarized in Table XII.

TABLE XII

DETERMINATION OF CARBON DIOXIDE

Oxidation Number	Temperature, °C.	Time, hr.	Total Carbon,[a] mg.	Total Carbon Dioxide, mg.
44	140	6	30.77	112.82
27	140	20	58.66	215.09

[a] Determined by radioactivity measurements.

It may be concluded from the data in Table XII that carbon dioxide is produced in considerable amounts during the oxidation of glucose. These results may be somewhat low, however, due to slight leaks which were detected at the inlet and

outlet valves of the bomb used as the reaction container. It is difficult to predict the effect of these leaks on the carbon dioxide determination without any means of monitoring the pressure in the bomb during a reaction. The manner in which carbon dioxide is formed will be discussed during the presentation of the oxidation scheme.

Nonvolatile Products

In order to determine if carbon dioxide was the only volatile carbon compound produced during the reaction, it was desired to compare the amount of carbon dioxide formed with the amounts of nonvolatile compounds remaining in solution. However, it was not feasible to analyze each solution quantitatively for all of the nonvolatile components. As a measure of these compounds, the total amount of nonvolatile carbon in solution was determined by a radioactive counting technique. This work was performed with the solutions from the reactions conducted at 140°C. The measurements determined only the amount of carbon in solution and in the case of Solutions 42 and 27, did not include the carbon content of the water-insoluble material presumed to originate from the polymerization of furfural-type compounds. The data obtained from this work are reported in Table XIII and are presented graphically in Fig. 6.

From the graphical presentation in Fig. 6, it appears that the nonvolatile carbon content of the reaction solutions is a linear function of the reaction time up to approximately twelve hours; thereafter, the carbon content decreases faster than would be expected from the earlier behavior. However, the carbon content measured was that in solution only, and did not take into account the carbon content of the precipitated material found in the sixteen- and twenty-hour reaction solutions. The amounts of insoluble material present in these solutions were not determined because of the difficulty of removing them from the reaction

container in any manner approaching quantitative transfer. It is believed, however, that the formation of this material is the primary cause of the deviation of the data from linearity at the longer reaction times.

TABLE XIII

NONVOLATILE CARBON CONTENT OF REACTION SOLUTIONS AT 140°C.

Oxidation Number	Time, hr.	Initial Carbon, g.	Carbon in Solution,[a] g.	Initial Carbon Determined, %
39	4	2.0000	1.9215	96.1
40	8	2.0000	1.8314	91.6
41	12	2.0000	1.7481	87.4
42	16	2.0000	1.6334[b]	81.7
27	20	2.0000	1.2786[b]	64.0

[a]The average of duplicate determinations is reported. The agreement of the individual values was within 0.15 mg.

[b]The amount of carbon in the precipitated material is not included in the reported value.

Comparison of Volatile and Nonvolatile Products

If it is assumed that the nonvolatile carbon content of the solutions is a linear function of the reaction time, there would be approximately 420 mg. of volatile carbon products (in mg. of carbon) from the twenty-hour reaction. The amount of carbon dioxide determined from this reaction was 58.7 mg. (expressed as mg. of carbon), which is far less than that required were it assumed that carbon dioxide was the only volatile product. This value was regarded with some skepticism because slight pressure leaks were detected around the bomb head.

Oxidation 44 was specifically conducted to resolve the discrepancy between the total amount of volatile carbon and that accounted for as carbon dioxide.

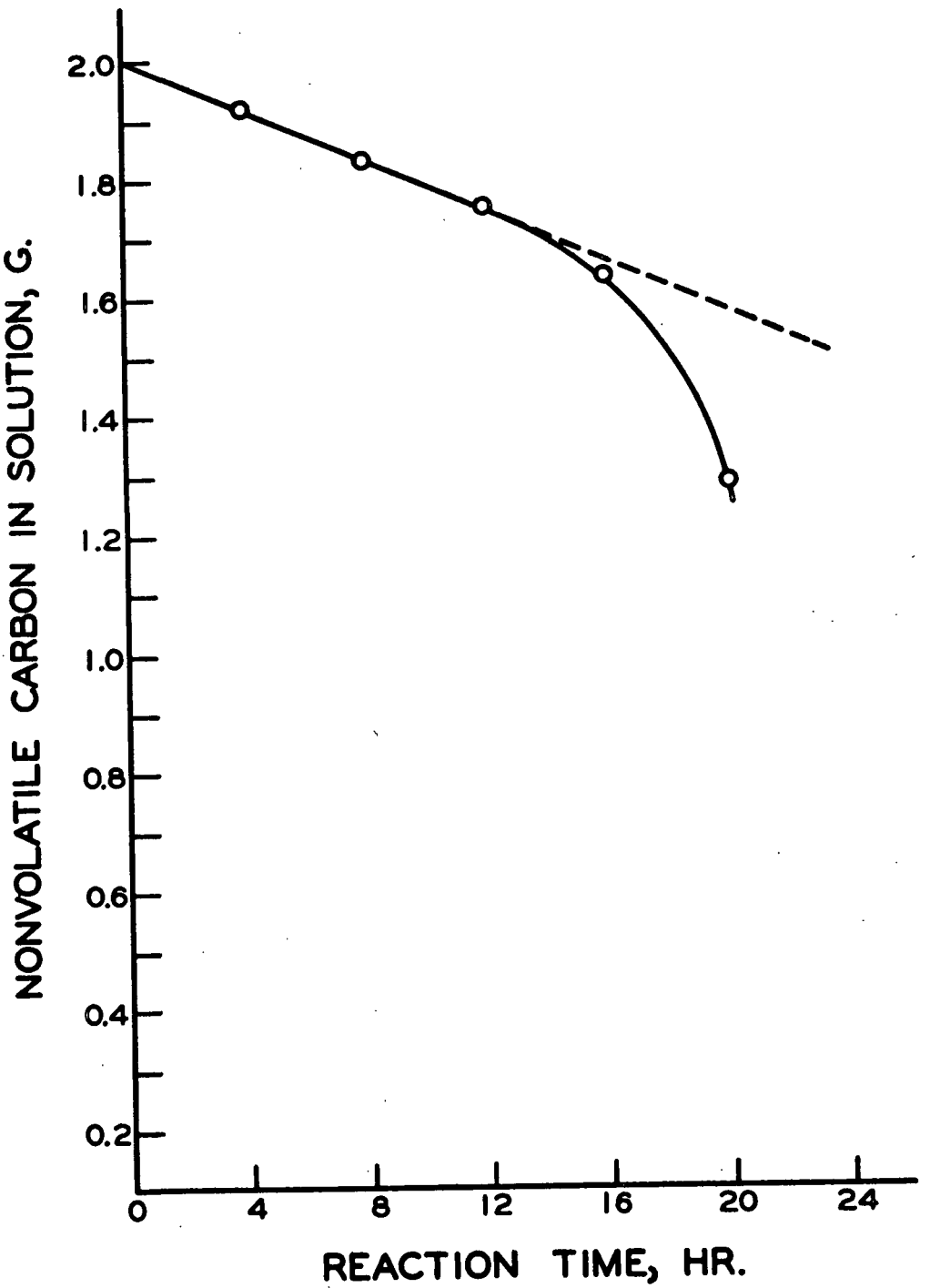


Figure 6. Nonvolatile Carbon Content of Reaction Solutions at 140°C.

The reaction time was six hours at 140°C. Although precautions were taken to prevent pressure leaks, slight leaks were still detected at the inlet and outlet valves on the bomb head. After the reaction had been terminated, the amounts of carbon dioxide produced during the reaction and of nonvolatile carbon in solution were measured.

The amount of carbon dioxide (expressed as mg. of carbon) found in the relief gas was approximately 30 mg. With reference to Fig. 6, one would expect to obtain about 130 mg. of carbon in this determination were carbon dioxide the only volatile product.

It was shown earlier that formaldehyde is not a product of the reaction. In addition, evidence was also presented which indicates that formic acid is not a major oxidation product of glucose. Some formic acid may be present, particularly at the longer reaction times, due to the oxidation of 5-hydroxymethylfurfural and furfural. These results when considered together with the nature of the other products previously identified make it difficult to conceive of other major volatile carbon products. The difficulty of proving that carbon dioxide is the major volatile product from the oxidation of glucose appears, at least in part, to reside in the equipment used to conduct the reactions.

When the bomb was opened upon completion of the carbon dioxide determination in Oxidation 44, the solution was found to contain some of the insoluble, brown material noted in the sixteen- and twenty-hour solutions. Evidently, during the period used for the carbon dioxide determination (307.5 hr.), the 5-hydroxymethyl-furfural and furfural formed during the reaction were oxidized and the products subsequently polymerized to yield the precipitated material. Such reactions are known to occur with furfural at room temperature in the presence of oxygen (58).

This phenomenon was not observed in the four-, eight-, or twelve-hour reactions in which the pressure was relieved rapidly after the reaction had been terminated.

It was not anticipated that the value obtained for the amount of nonvolatile carbon in solution would agree with the value of 1.87 g. predicted from Fig. 6 due to the presence of the insoluble material. The amount of carbon in solution was found to be 1.75 g.

PEROXIDE COMPOUNDS

It was shown earlier that the initial product formed in the free-radical oxidation of an organic compound by molecular oxygen is a hydroperoxide, ROOH. These compounds are produced during the cyclic propagation sequence. Their subsequent reactions lead to the more stable compounds observed as reaction products.

Church (24) was not able to detect peroxide compounds among the reaction products of crystalline glycosides oxidized by molecular oxygen. He concluded that the temperatures used in his study (108-130°C.) caused the immediate decomposition of these products and precluded their detection. Radchenko, et al. (71) have studied the peroxides formed during the oxidation of cellulose acetate. The results of their work suggested that three types of structurally different peroxides were present, some of which were stable up to 140°C. Unfortunately, no further details regarding the nature of the compounds were given in the abstract of the article.

The titanium sulfate test (72) was used to investigate several of the reaction solutions at each temperature for the presence of peroxide compounds. A yellow color is obtained when this reagent* is added to a solution containing

*The reagent consists of a solution of titanium sulfate in 50% sulfuric acid.

hydrogen peroxide. Organic peroxides such as benzoyl peroxide or peracetic acid yield an orange color which was observed to darken somewhat with time.

Since the reaction solutions in the present study varied in color from light yellow to amber, it was difficult to discern any initial change in color of these solutions upon addition of the reagent. The color of the solutions gradually darkened with time in a manner similar to those of benzoyl peroxide and peracetic acid. It was observed qualitatively that solutions to which only 50% sulfuric acid was added did not darken as much as those to which the reagent was added.

In order to circumvent this difficulty, aliquots were tested from the neutral fractions of the oxidations conducted at 140°C. These natural fractions, which were obtained by separation of the reaction solutions into neutral and acidic parts with ion-exchange resins, were colorless. Each solution gradually developed an orange color after the reagent had been added. Control solutions to which only 50% sulfuric acid was added did not develop any color. These observations are interpreted as evidence for the presence of peroxide compounds in the reaction solutions.

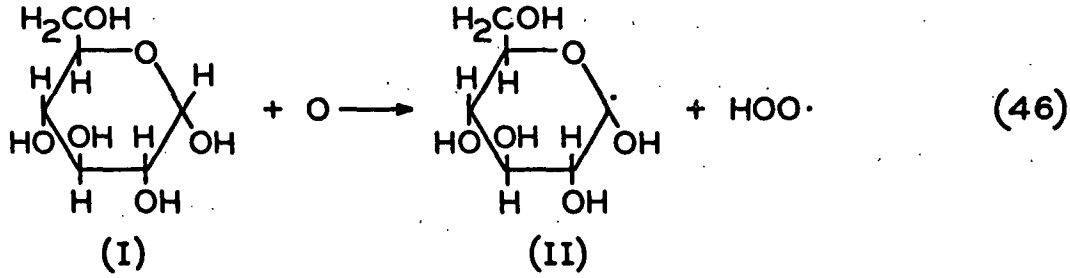
A POSTULATED MECHANISM FOR THE OXIDATION OF GLUCOSE

The following pathway for the oxidation of glucose in aqueous solution by molecular oxygen is proposed based on the experimental results obtained in this work together with those obtained from other investigations which proceed by a free-radical mechanism and in which molecular oxygen is the oxidant. The oxidative scheme involves the one-carbon, stepwise degradation of glucose to products containing fewer carbon atoms. Some of the experimental data indicates that two-carbon degradative reactions also occur. However, the data are not sufficient to discuss the manner in which these latter reactions proceed.

Phillips and Criddle (82) have investigated the ultraviolet irradiation of aqueous sorbitol solutions in the presence of oxygen. Their results showed that sorbitol underwent stepwise degradation from both ends of the molecule. The initial products were hexoses. Thereafter, the degradation proceeded by way of hexonic acids to pentoses. During irradiation periods of up to six hours, the amount of sorbitol reacted could be accounted for by the amounts of hexoses, hexonic acids, and pentoses formed. The amount of carbon dioxide produced indicated that the pentoses were formed by decarboxylation of the hexonic acids. A similar reaction sequence has been proposed for the formation of arabinose from glucose which was produced during the photochemical degradation of cellulose in the presence of air and water (83). 2-Ketogluconic acid was thought to be the precursor of arabinose.

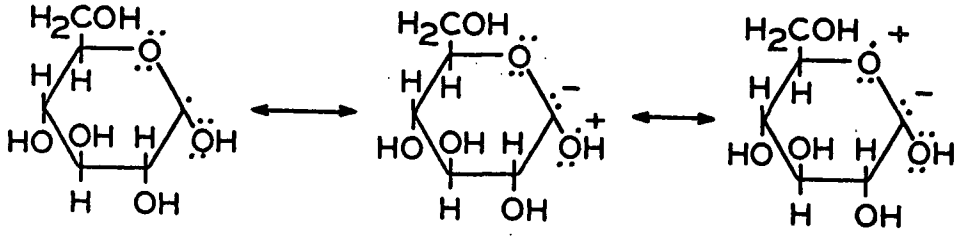
INITIATION

The aldehyde or hemiacetal group is the most readily oxidized group in carbohydrates (73). From the discussion presented earlier regarding the factors which determine the position of initial attack in free-radical reactions, it is expected that the initial attack of a free radical on glucose would occur primarily at the anomeric carbon-hydrogen bond. Experimentally, gluconic acid is found to be the major stable reaction product at low degrees of reaction. This fact together with the absence of glucosone indicates that the initial attack of molecular oxygen is principally at carbon atom one.



(I) (II) (46)

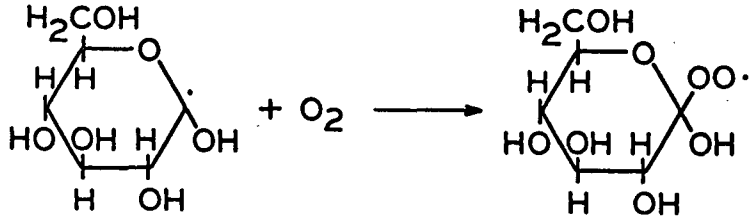
The radical, (II), formed in Equation (46) is stabilized by resonance to a greater extent than those formed by the abstraction of a hydrogen atom from any of the remaining carbon atoms due to the availability of unshared electrons on the two oxygen atoms adjacent to the anomeric carbon atom. Since each of the other carbon atoms is adjacent to only one oxygen atom, the number of possible resonance forms is decreased. Species (II) is stabilized as follows:



(47)

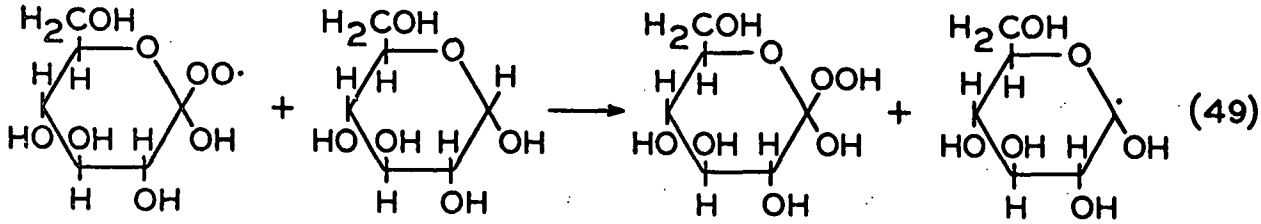
PROPAGATION

After the reaction has been initiated, Species (II) enters the cyclic chain propagation sequence as illustrated in Equations (48) and (49).



(48)

(II) (III)

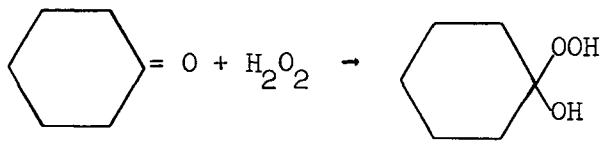


(49)

(III) (I) (IV) (II)

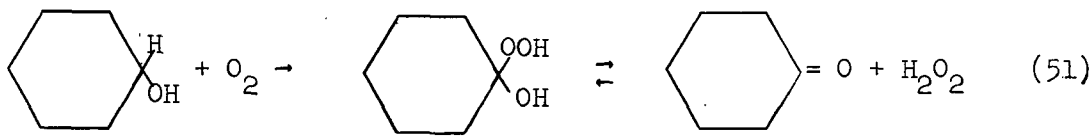
The reaction of Species (II) with molecular oxygen proceeds very rapidly to yield Species (III). Species (III), in turn, abstracts a hydrogen atom from the anomeric carbon atom of glucose to yield Species (IV) and regenerate Species (II), which then reenters the propagation sequence in Equation (48).

It is seen that the initial product of the reaction is the hydroperoxide, Species (IV), produced in Equation (49). Compounds structurally similar to Species (IV) have been prepared by Milas, et al. (74). These workers isolated 1-hydroperoxycyclopentanol and 1-hydroperoxycyclohexanol in addition to other peroxides prepared by the action of hydrogen peroxide on cyclopentanone and cyclohexanone, respectively. The reaction is illustrated with the latter compound in Equation (50).



(50)

Brown, et al. (75) have prepared 1-hydroperoxycyclohexanol by the liquid-phase free-radical oxidation of cyclohexanol with molecular oxygen. The temperature range used in their work was from 110 to 140°C.

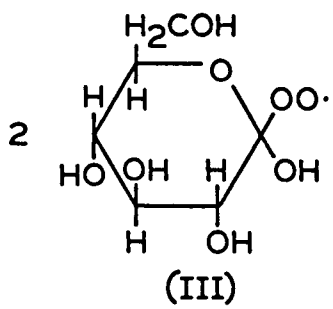


(51)

These investigators have found that 1-hydroperoxycyclohexanol is the initial product of the reaction. The other peroxides noted were the result of further interactions between this compound and cyclohexanone and hydrogen peroxide, which are also formed as indicated in Equation (51).

TERMINATION

By analogy to the free-radical oxidation of hydrocarbons discussed earlier, the addition of oxygen to Species (II) in Equation (48) is thought to occur rapidly. In most olefin oxidations, termination reactions such as the combination of two R· radicals or one R· and one ROO· radical have been found to be negligible above an oxygen pressure of 0.13 atm. (33-34). In the present case due to the high oxygen pressure, termination probably results from the combination of two radicals of the type illustrated by Species (III), which subsequently rearrange to yield inactive products.



(III)

$$\longrightarrow \text{INACTIVE PRODUCTS} \qquad (52)$$

Termination reactions, which account for only a fraction of the total products, are not well understood. There is evidence that the process includes the evolution of oxygen; however, the actual reaction mechanism seems to differ from radical to radical. Russell (42) has proposed a cyclic process for the termination of reactions in which the radical contains an alpha-hydrogen atom.

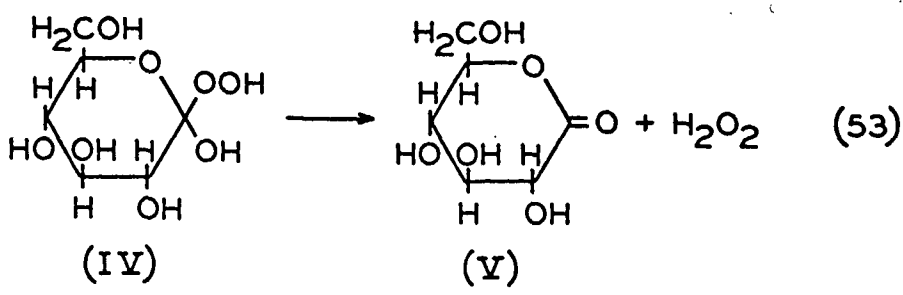
FORMATION OF OXIDATION PRODUCTS

Since chain-terminating reactions account for only a minor fraction of the oxidation products, most of the products are derived from further reactions of the hydroperoxide compounds formed in the propagation sequence. Alkyl and acyl hydroperoxides are of limited value as sources of free radicals, since they tend

to decompose to molecular rather than radical products. These reactions are considered in the following sections with respect to the oxidation products determined in this work.

Gluconic Acid

The hydroperoxide, Species (IV), formed from the oxidation of glucose, may react as shown in Equation (53).

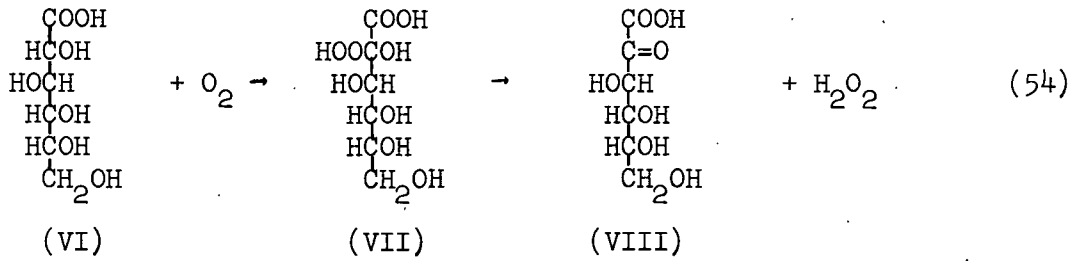


(53)

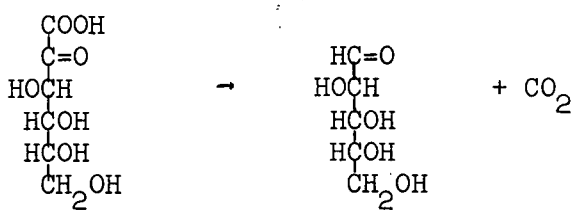
This reaction is analogous to that shown in Equation (51) for the hydroperoxide formed from the oxidation of cyclohexanol. The oxidation of other secondary alcohols has also been shown to proceed via a hydroperoxide intermediate to the corresponding ketone and hydrogen peroxide (76-77). The "ketone" produced in Equation (53) is the delta-lactone of gluconic acid. The latter compound was found to be the primary oxidation product in this work.

Arabinose

The product analysis data presented earlier indicates that arabinose is not formed directly from glucose but is a secondary oxidation product and is formed from gluconic acid. Since 2-ketogluconic acid was detected among the reaction products, it seems likely that arabinose could be formed, at least in part, from gluconic acid via 2-ketogluconic acid as illustrated in the following reactions. (For convenience and clarity, the straight-chain formulae rather than the Haworth structures are shown.)



(VI) (VII) (VIII) (54)



(VIII) (IX) (55)

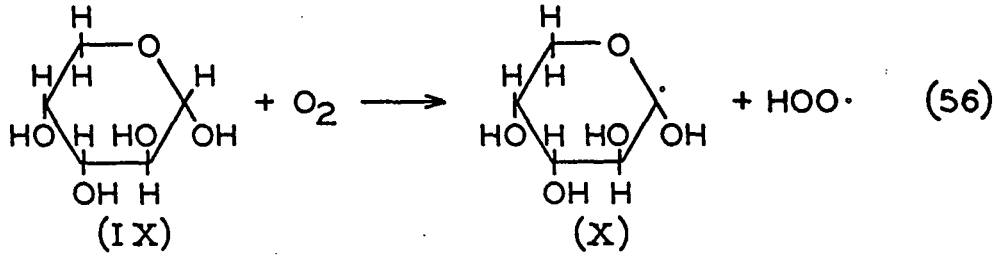
If the reaction conditions are severe enough, almost any carboxylic acid, RCOOH, may be made to undergo decarboxylation. However, decarboxylation occurs most readily if, within group R, there is a strong electron-attracting substituent such as a carbonyl, cyanide, or nitro group (78). Acids containing these substituents within the group R decarboxylate readily at temperatures from 100 to 150°C. (79). It appears that this type of reaction may occur in the present system.

The cleavage of the C1-C2 bond in gluconic acid may also occur with the direct formation of arabinose and carbon dioxide. Although the exact mechanism of the reaction is not known, the Ruff degradation of aldonic acids, a free-radical reaction, proceeds in this manner (80). The perhydroxyl radical, HOO·, is thought to be the reactive species. This radical is formed in the postulated initiation reaction.

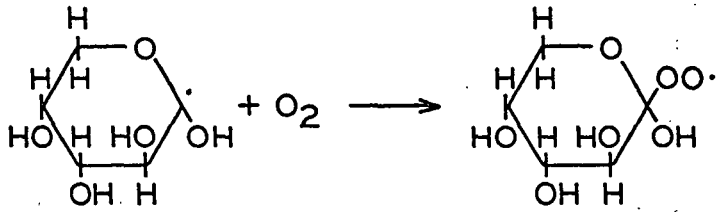
Arabonic Acid

The formation of arabonic acid by the oxidation of arabinose with molecular oxygen would be expected to proceed in an analogous manner to the formation of

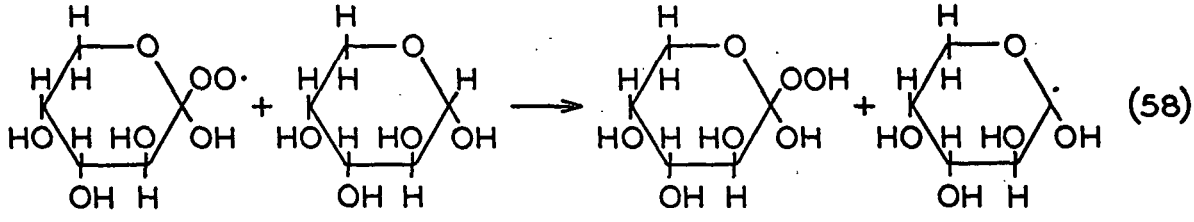
gluconic acid from glucose. The free radical, (X), produced in the initiation step would also be stabilized as shown in Equation (47).



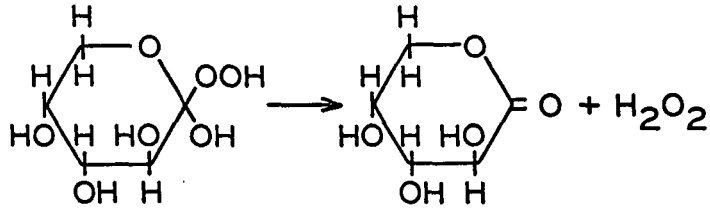
(IX) (X) (56)



(X) (XI) (57)



(XI) (IX) (XII) (X) (58)



(XII) (XIII) (59)

From the earlier discussion regarding the time-dependent concentrations of arabinose and arabonic acid, it was concluded that some arabonic acid was formed from gluconic acid in addition to that formed from arabinose. Since the direct cleavage of the C1-C2 bond in gluconic acid would yield arabinose and carbon dioxide, it is more likely that arabonic acid is formed from gluconic acid via 2-ketogluconic

acid. The free-radical degradation of 2-ketoaldonic acids to aldonic acids of one less carbon atom is known to occur (73). Again, the perhydroxyl radical, HOO·, is thought to be the reactive species.

Other Oxidation Products

Erythrose and erythronic acid were identified as oxidation products by paper chromatography. From the preceding discussion, it would be expected that erythrose and erythronic acid would be formed from arabonic acid similarly to the formation of arabinose and arabonic acid from gluconic acid. Additionally, erythronic acid would also be the primary oxidation product of erythrose.

The presence of oxalic acid among the oxidation products early in the reaction indicates that some cleavage of the C2-C3 bond in glucose occurs with the formation of two- and four-carbon compounds. Since oxalic acid, erythrose, and erythronic acid were not studied in detail, it is not possible to speculate on the nature of the products formed by the rupture of the C2-C3 bond.

EXPERIMENTAL EQUIPMENT AND PROCEDURES

EQUIPMENT

OXIDATION APPARATUS

The oxidations of glucose in aqueous solution were conducted in the apparatus shown in Fig. 7. It consists of an oven modified to permit magnetic stirring of the reaction solutions, the associated temperature control equipment, and a container for the reaction solutions. These items of equipment are discussed in the following paragraphs.

Oven and Controls

The oven was a common Thelco laboratory model modified to allow the installation of the equipment shown in Fig. 7. Accurate temperature control was obtained by using a knife heater immersed directly in the oil bath. With this modification, the function of the oven was reduced to that of an insulated container on which the temperature control and stirring equipment for the bath was mounted.

The oil bath in which the bomb was placed consisted of a 3000-ml. stainless steel beaker containing Dow Corning 550 Silicone Fluid. The level of the oil within the beaker was adjusted to avoid contaminating the valves on the bomb head. A stainless steel shelf was used to support the oil bath.

Temperature control was provided by a thermometer immersed in the oil and connected to a Thermocap relay[1]. Two thermometers, graduated in 0.1°C. increments, were used in this study; each was calibrated by comparison with a set of thermometers calibrated at the National Bureau of Standards. During a reaction the temperature of the bath varied by ± 0.15°C. about the desired temperature.

[1]Niagara Electron Laboratories, Andover, New York.

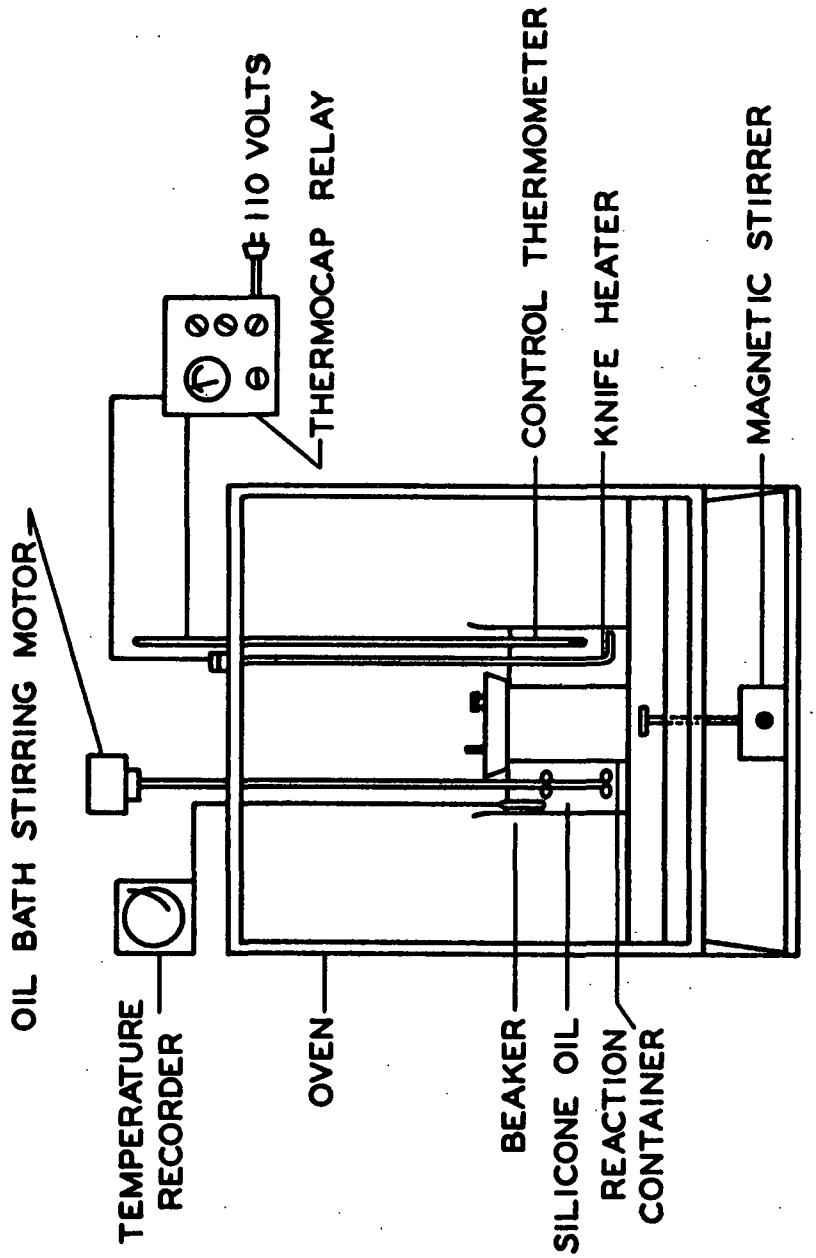


Figure 7. Oxidation Apparatus

The reaction temperature was recorded continuously during each oxidation with a Dickson Minicorder[2]. Although the recorder is not particularly sensitive to small temperature fluctuations, it was used to insure that there were not any large deviations from the desired temperature during the course of a reaction.

The oven was modified to permit stirring of the oxidation solutions. The motor of a magnetic stirrer was inverted in its casing and fitted with an extended stirring shaft. The stirrer was mounted beneath the oven with the shaft protruding up into the oven through a hole drilled in the bottom. The bar magnet was mounted on the end of the shaft. In order to fix the alignment of the motor, the casing was recessed in a sheet of plywood fastened to the oven legs.

Reaction Container

A Parr No. 1101 double valve oxygen bomb[3] was used as the container for the reaction solutions. The bomb was fitted with a custom-made Teflon insert, machined from a Teflon rod, into which the solutions were placed to avoid metal ion catalysis. Generally, the neoprene gaskets in the bomb were replaced with Teflon gaskets because the reaction temperatures caused deterioration of the former. It was necessary to use a pipe wrench to tighten the screw cap of the bomb head in order to obtain a good seal. However, due to the difficulty of obtaining a good seal at the inlet valve with a Teflon gasket, a neoprene gasket was used. It was necessary to replace this gasket after each oxidation. The needle-type gas outlet valve permitted relief of the oxidation gases at any desired rate.

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[2]The Dickson Company, 7420 Woodlawn Avenue, Chicago, Illinois.

[3]Parr Instrument Company, 211 53rd Street, Moline, Illinois.

Oxygen and Charging Apparatus

The oxygen used in the reactions was Matheson Extra Dry Grade[4]. The purity was quoted in their catalog as 99.6% minimum and the dew point as less than -70°F.

The gas charging apparatus was obtained on loan from the Analytical Chemistry Group at The Institute of Paper Chemistry. A new pressure gage, obtained from Parr Instrument Company, was calibrated according to the method of Church (24). The true pressure was found to be nearly a linear function of the gage pressure. The calibration data were used during all charging operations.

MEASUREMENT OF RADIOACTIVITY

The kinetic data and a major portion of the product analysis data were obtained by measurements of radioactivity. The method employed involved converting the sample to carbon dioxide and measuring the activity of the latter compound.

Thomas-Van Slyke Manometric Apparatus

The Thomas-Van Slyke apparatus was originally designed to determine the amount of carbon in an organic compound from pressure – volume – temperature measurements after the sample had been oxidized to carbon dioxide. A later modification of the apparatus permitted carbon determinations by the use of carbon-14 in conjunction with gas-phase proportional counters.

The apparatus and technique are thoroughly described in the original papers (88-91). Briefly, the organic sample is oxidized to carbon dioxide and the latter is absorbed in an aqueous solution of sodium hydroxide and hydrazine sulfate. After the extraneous gases have been removed from the absorption chamber, the carbon dioxide is liberated by the addition of lactic acid. From

[4]The Matheson Company, P. O. Box 966, Joliet, Illinois.

the measurements of its pressure and temperature at a fixed volume, the amount of carbon dioxide is calculated. That portion of carbon dioxide which is radioactive may be determined by transferring the gas to a Bernstein-Ballentine counting tube with methane and recording the activity of the sample with a suitable scaling unit. In this study a Nuclear-Chicago Model 182 scaling unit was employed for counting.

Bernstein-Ballentine Counting Tubes

Before a counting tube may be used, the optimum operating voltage and the fraction of the tube which is effective in counting must be determined. The latter quantity, V_r, corresponds to that fraction of the total volume of the tube which is silvered. It was determined by filling the tube to the appropriate levels with toluene and weighing as outlined by Bernstein and Ballentine (92). The value of V_r for the tube used in this study was 0.837.

The optimum operating voltage of a tube is determined by transferring a sample of radioactive carbon dioxide to the tube and establishing a curve of counts per minute versus voltage over the voltage range in which counting occurs. The plateau region in the curve corresponds to the voltage range in which all of the disintegrations occurring within the tube are recorded. The optimum operating voltage is taken as the voltage at the midpoint of the plateau region. For carbon dioxide – methane mixtures, this voltage should be approximately 3800 volts or in the range of 3600-4000 volts (90-91). The optimum operating voltage of the tube used in this study was 3800 volts.

Since only the silvered fraction of the tube is effective in registering the disintegrations within the tube, a correction factor, E, must be used to obtain the total number of disintegrations. Van Slyke, et al. (90) found that,

for tubes containing 7 mg. of active carbon or less, E was 0.98. This value was used in the calculations in this study since the samples oxidized in the Van Slyke apparatus contained from 2.0 to 3.5 mg. of active carbon.

PROCEDURES

PREPARATION OF STARTING MATERIALS

Glucose

The glucose used in this study was labeled with carbon-14 in order to facilitate analysis of the solutions after reaction. It was prepared by diluting uniformly labeled glucose of high specific activity with inactive glucose. The dilution factor was approximately 6000. The preparative procedure is described in the following paragraphs.

The labeled material* was obtained freeze-dried and vacuum-sealed in glass ampules, each containing 0.05 millicuries (mc.) of glucose of specific activity 3.9 mc./mM. Initially, a quantity of labeled glucose was prepared by transferring the contents of one ampule (0.05 mc.) to a 1000-ml. round-bottom flask by repeated washing of the ampule with distilled water. One hundred and fifty grams of inactive α-D-glucose were added to the flask and the resultant solution concentrated under reduced pressure to an immobile sirup.

The sugar was crystallized as described by Isbell, et al. (85). Purified methanol (5 ml. total) was added slowly with mixing to give a sirup of reasonable mobility. Pure isopropyl alcohol (0.7 ml.) was then added dropwise with mixing to incipient turbidity. The solution was seeded and set aside to crystallize for twelve hours. After the product had been removed from the flask, it was washed with absolute ethanol and dried in vacuo at 60°C. for twenty-four hours.

*The labeled glucose was purchased from Nuclear-Chicago Corporation, 333 East Howard Avenue, Des Plaines, Illinois.

A yield of 146.5 g. (97.7%) was obtained. The melting point was 144-145°C.; literature: 146°C. The equilibrium specific rotation, $[\alpha]_D^{23}$, was + 52.2° (water); literature: + 52.7° (water). The specific activity was 1846 disintegrations per minute per milligram of active carbon.

Preliminary experimental work showed that the specific activity of the labeled glucose prepared initially was too low for satisfactory analysis of the oxidation products by isotope dilution. It was increased by the addition of 0.45 mc. of glucose, specific activity 3.9 mc./mM, to 140.2 g. of the product prepared previously. The procedure was the same as that in the preceding paragraphs. A yield of 137.7 g. (98.1%) of glucose was obtained which melted at 142.3-144.3°C. The equilibrium specific rotation, $[\alpha]_D$, was + 52.7°. The specific activity had been raised to 19,272 disintegrations/minute/milligram of active carbon.

The purity of the labeled starting material was also checked by paper chromatography in three solvent systems (Systems A, B, and C, Appendix IV). One thousand microgram quantities were spotted on Whatman No. 1 paper. After development the papers were treated with silver nitrate reagent. In Systems A and B a very nearly imperceptible spot was noted in the disaccharide region in addition to that given by glucose. Only glucose was detected on the chromatogram run in System C. Since silver nitrate reagent is very sensitive and can be used to detect microgram quantities of sugars, the indicated purity of the glucose is approximately 99.9%.

Water

The water used to make up the oxidation solutions was prepared according to the method of Bauer and Lewin (86). Distilled water was redistilled from an alkaline permanganate solution followed by a second distillation from dilute

sulfuric acid solution. The specific resistance, measured with a Solu-Bridge Model RD 146 meter, was 0.52 megohm·cm. The specific conductance was 1.92×10^{-6} mhos·cm.$^{-1}$.

OXIDATION PROCEDURE

The following procedure was used to conduct each oxidation. Five grams of glucose were weighed in an aluminum dish and transferred to a 100-ml. volumetric flask. The weighing dish was washed with water several times to insure the complete transfer of all glucose. After the solution had been diluted to volume, it was transferred to the Teflon insert within the bomb, a Teflon-coated stirring bar added, and the bomb sealed. It was charged with oxygen to a pressure equivalent to 50 atm. at 25°C. (In each oxidation the amount of oxygen present initially was constant and equal to 0.343 mole.)

The bomb was positioned in the oil bath which had been preheated to the desired reaction temperature, and the magnetic stirrer started. Zero time was taken as the time the reaction temperature was reestablished. The times required were 50, 90, and 125 minutes at the respective reaction temperatures of 110, 125, and 140°C. These time intervals were reproducible to within $\pm$ 3 minutes.

After the desired reaction period had elapsed, the reaction was terminated by removing the oil bath from the oven and placing the bomb in an ice-water bath for one-half hour.

ANALYTICAL METHODS

The primary methods employed for the analysis of the oxidation solutions were isotope dilution, descending paper chromatography, and ultraviolet spectrophotometry. In addition, other methods of less general applicability were used

to obtain information regarding specific reaction products. The experimental techniques are described in the following sections in conjunction with the particular compound with which they were used.

Nonvolatile Compounds

Glucose

The decrease in the amount of unreacted glucose with time at each reaction temperature was determined by the technique of isotope dilution. The glucose was isolated as a pentaacetate derivative. The latter compound was prepared by the general method of Wolfrom, et al. (87) with some modifications.

A known amount of inactive glucose was added to a 25-ml. aliquot of the reaction solution and the solution concentrated to a sirup under reduced pressure. Acetic anhydride and sodium acetate were added to the flask containing the sirup. The flask was then placed in a boiling water bath for three hours. After the flask had been removed from the bath and cooled to room temperature, crushed ice was added with stirring. The product, β-D-glucose pentaacetate, crystallized from the acetylation solution. It was filtered and washed with cold water. Purification was effected by recrystallization from 95% ethanol. The product was recrystallized until a constant specific activity was obtained. Generally, four to five recrystallizations were necessary. The melting point of the final product was typically 129-130.5°C.; literature value: 132°C. (87).

From the specific activity of the starting glucose, the amount of inactive glucose used for dilution, and the specific activity of the final product, the amount of unreacted glucose remaining after the reaction had been conducted was calculated. A description of the technique of isotope dilution including a sample calculation is presented in Appendix I.

Gluconic Acid

The analyses of the reaction solutions for gluconic acid were performed by the technique of isotope dilution. The phenylhydrazide derivative was investigated, and it proved to be both easily prepared and purified. During the preliminary work, the acid was isolated as its calcium salt. However, purification of small amounts of the latter compound was difficult.

A 15-ml. aliquot of the reaction solution was diluted with inactive glucono-1,5-lactone. The dilution factor was approximately one hundred based on estimates from paper chromatography of the amount of gluconic acid present. The resultant solution was titrated with standardized sodium hydroxide solution to pH 10 in a constant temperature bath at 50°C. in order to convert the active and inactive acids to their sodium salts. Ion-exchange resins were then used to separate the titrated solution into a neutral and an acidic fraction. The fractionation procedure is described in Appendix III.

Gluconic acid phenylhydrazide was prepared from the acidic fraction by the procedure of Fischer and Passmore (93). After the acidic fraction had been concentrated under reduced pressure to a ten percent solution based on the inactive lactone added, a 50% acetic acid – water solution, equal in volume to that of the concentrated acidic fraction, was added. One part phenylhydrazine was added next and the solution placed in a boiling water bath. Usually, after three hours, the product had partially crystallized. The mixture was cooled to room temperature. After standing for several hours, the phenylhydrazide was filtered, washed in order with cold water, absolute ethanol, and ether. It was dried *in vacuo* at 50°C. The crude product was recrystallized to constant specific activity from hot water. Generally, three recrystallizations were required. Typically, the purified phenylhydrazide melted at 200-201.5°C.; literature value: 200-201°C. (93).

Arabinose and Arabonic Acid

Arabinose and arabonic acid were isolated from the reaction solutions as their diphenylhydrazone and phenylhydrazide derivatives, respectively.

Inactive arabinose and arabono-1,4-lactone were added to a 25-ml. aliquot of the solution to be analyzed. After titration of the solution with sodium hydroxide, it was separated into a neutral and an acidic fraction (see Appendix III).

D-Arabinose diphenylhydrazone was prepared according to the method of Mandl and Neuberg (94) from the neutral fraction. The aqueous fraction was added to boiling methyl alcohol containing sodium acetate. Subsequently, diphenylhydrazine hydrochloride was added. The product usually began to crystallize from solution in 5-10 minutes. The mixture was refrigerated for several hours after which the diphenylhydrazone was filtered and washed with 3% aqueous methanol and 5% ethanolic ether. It was recrystallized to constant specific activity from 2-methoxyethanol. The melting point was 203.5-204°C.; literature: 207-208°C. (94).

The procedure of Glattfeld (95) was used to prepare arabonic acid phenylhydrazide. The acidic fraction was evaporated under reduced pressure to a sirup in a tared flask. Phenylhydrazine and absolute ethanol were added in volumes (ml.) equal to the weight of the sirup (g.). The mixture was warmed with stirring on a steam bath until a homogeneous solution was obtained. It was then set aside to crystallize for approximately twelve hours. The product was filtered, washed with absolute ethanol, and recrystallized from hot water to constant specific activity. The melting point of the phenylhydrazide was typically 210-211.5°C.; literature value: 213°C. (95).

Glucosone

Several reaction solutions were examined for glucosone by comparing the amounts of unreacted glucose determined as glucose pentaacetate and as glucose phenylosazone. The method used to prepare the phenylosazone was that of Richtmyer (96).

Inactive glucose was added to a 25-ml. aliquot of the reaction solution which was subsequently diluted with water to a glucose concentration of 2 g./100 ml. Four molecular equivalents of phenylhydrazine and glacial acetic acid were added and the mixture heated on a steam bath for two hours. Upon cooling the phenylosazone was obtained. It was filtered and washed successively with 10% acetic acid, water, cold ethanol, and ether. Recrystallization was effected from twenty parts 50% pyridine – water. The phenylosazone melted at 205-206°C.; literature value: 208-209°C. (96).

Oxalic Acid

Oxalic acid was isolated directly from the reaction solutions as its dihydrate. Inactive anhydrous oxalic acid was added to an aliquot of the solution, which was then concentrated under reduced pressure to a thick sirup and refrigerated. After the crystalline dihydrate had been filtered and washed with cold water, it was recrystallized to constant specific activity from hot water. The melting point of the product was 98-101°C. after three recrystallizations; literature value: 99-101°C. (97).

5-Hydroxymethylfurfural

The amounts of 5-hydroxymethylfurfural (HMF) in the reaction solutions at each temperature were estimated from the ultraviolet spectra of the solutions as determined with a Beckman DK-2 recording spectrophotometer. The absorption maximum of HMF occurs at 284 millimicrons (62). The wavelength of the maximum

was near this value initially, but as the reaction time was increased at each temperature, the wavelength of the maximum decreased. This behavior is due to the formation of furfural from arabinose, which is a secondary reaction product. Furfural absorbs strongly at 277.5 millimicrons (62).

The amount of HMF in each solution was calculated from the following relationship based on Beer's Law and the known dilution of the particular solution for which the spectrum was determined.

$$A = abc \qquad (60)$$

where A = absorbance

a = absorptivity, $1.mg.^{-1}cm.^{-1}$

b = cell length, cm.

c = concentration, mg./l.

It was assumed that HMF was the only absorbing species present. Therefore, the absorptivity for this compound ($a = 0.134\ 1.mg.^{-1}cm.^{-1}$) was used in Equation (60). The calculated amount of HMF (see p. 29) for each oxidation is probably low since it does not include that which may have polymerized and yields the water-insoluble material noted at the longer reaction times at each temperature.

HMF was identified by the preparation and isolation of its 2,4-dinitrophenylhydrazone from one of the reaction solutions. The preparative method of Blanksma and Wackers (63) was used. Twenty-four milligrams of 2,4-dinitrophenylhydrazine in 5 ml. of ethanol were added to 50 ml. of Solution 28 (24 hours at 125°C.), which was estimated to contain 12 mg. of HMF. After the addition of several drops of concentrated sulfuric acid, the solution was heated to 90°C. and then cooled slowly to room temperature. The crystalline product was filtered and dried *in vacuo* at 50°C. The small amount of 2,4-dinitrophenylhydrazone was

not further purified. The melting point of the crude product was 183-185°C.; literature value: 184°C. (63).

Nonvolatile Carbon in Solution

The nonvolatile carbon contents of the solutions from the reactions conducted at 140°C. were measured. The measurements determined only the carbon in solution and in the case of Solutions 42 and 27 (16 and 20 hours, respectively) did not include the carbon content of the water-insoluble material presumed to originate from the polymerization of HMF and furfural.

The amounts of insoluble material in Solutions 42 and 27 were not measured. Although some of the material was present in the solutions after the latter had been transferred from the reaction containers, much of it adhered to the walls of the Teflon inserts and to the Teflon-coated stirring bars and could not be removed in any manner approaching quantitative transfer.

The general technique used to determine the total nonvolatile carbon in each solution was based on radioactivity measurements. An aliquot of each solution, calculated to contain from 2.0 to 3.5 mg. of carbon per 2 ml. of solution when diluted to a particular volume, was transferred to the appropriate volumetric flask and diluted to volume with water. Duplicate 2-ml. samples from each diluted solution were measured into separate combustion tubes with a two-milliliter stopcock pipet. The solutions were then concentrated to dryness under reduced pressure on a rotary evaporator at 30°C. and stored for 10-12 hours in vacuo over phosphorus pentoxide in a desiccator. The total activity of the sample was measured with the Van Slyke apparatus. From the initial specific activity and the known dilutions, the total amount of carbon in solution was calculated. The data obtained in this manner were reported in Table XIII, p. 50.

Erythrose, Erythronic Acid, and 2-Ketogluconic Acid

Erythrose, erythronic acid, and 2-ketogluconic acid were identified as reaction products by descending paper chromatography. The mobilities of these compounds in various developers were compared with those of authentic samples. The developers and spray reagents used in this work together with the R_g values of the various reaction products are presented in Appendix IV.

Volatile Compounds

Carbon Dioxide

The carbon dioxide produced during the oxidation of glucose was determined by utilizing its radioactivity. After the reaction had been terminated by rapidly cooling the bomb in an ice-water bath, the gas was relieved through the absorption apparatus shown in Fig. 8.

The bomb was placed on a magnetic stirrer and connected in series to a water vapor trap, containing a dry ice-alcohol mixture, and two carbon dioxide absorption bottles, each containing 50 ml. of 3N sodium hydroxide solution. After the gas within the bomb had been relieved, nitrogen was passed through the system until a constant specific activity was obtained from the sodium hydroxide solution in the first absorption bottle.

The specific activity measurements were made with the Van Slyke apparatus. Periodically, the nitrogen flow was stopped and a 1-ml. sample withdrawn from the first absorption bottle. After the sample had been transferred to the Van Slyke apparatus, the carbon dioxide was liberated by the addition of 5N lactic acid and transferred to a Bernstein-Ballentine counting tube. The activity was recorded with the scaling unit. From the activity of the aliquot, the total activity within the absorption bottle was calculated. The amount of carbon

dioxide produced during a reaction, as milligrams of carbon, was calculated by dividing the total activity by the specific activity of the starting material.

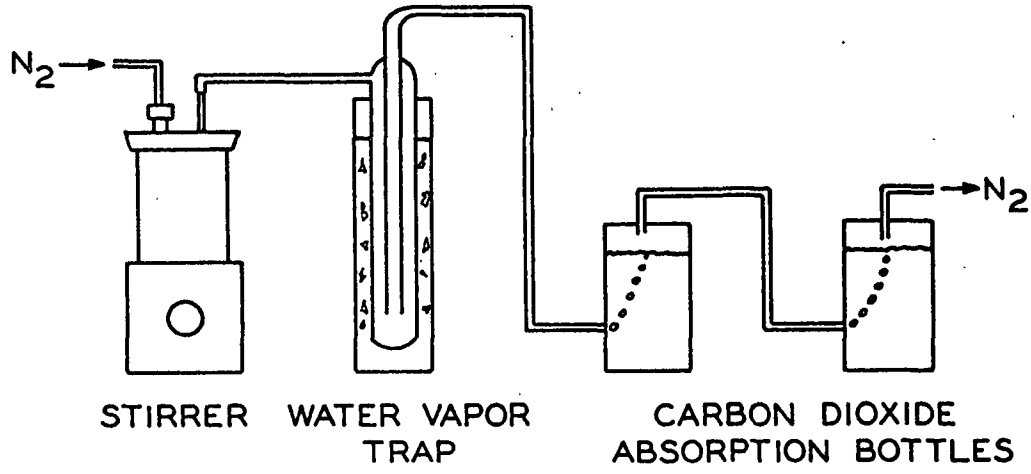

Figure 8. Carbon Dioxide Absorption Apparatus

During the preliminary work, the technique was shown to be accurate to within 5%. In all cases, a negligible amount of carbon dioxide reached the second absorption bottle.

Formaldehyde and Formic Acid

Formaldehyde and formic acid were not products from the oxidation of glucose. Dimedone was used to investigate the reaction solutions for the presence of formaldehyde. Although there was evidence for the presence of formic acid in some of the reaction solutions, the positive identification of this compound by derivatization was unsuccessful. The p-bromophenacyl ester was used as the characteristic derivative.

The techniques used to detect these compounds were summarized earlier on p. 43 and p. 45, respectively.

CONCLUSIONS

D-Glucose in aqueous solution is oxidized by molecular oxygen. In the temperature range from 110 to 140°C., the nonautocatalytic reaction is well described as being of order 1.5 with respect to the concentration of glucose up to approximately 35-40% reaction. The energy of activation in this temperature range is 26.8 kcal./mole, which is comparable with the activation energies of similar reactions. The observed reaction order and other evidence from this study indicate that a free-radical chain mechanism involving direct initiation of the reaction by molecular oxygen is operative.

The deviation of the data at higher degrees of reaction from the oxygen-initiated kinetic scheme is attributed to the formation of 5-hydroxymethylfurfural from glucose in a series of dehydration reactions which are competitive with those by which glucose is oxidized. Although the formation of 5-hydroxymethylfurfural was not studied in detail, the experimental data indicate that a large amount of glucose, in addition to that oxidized to gluconic acid, is consumed in these reactions above approximately 35-40% reaction. An important factor in explaining this behavior appears to be the acidity developed during the reaction.

The oxidation products which were identified include gluconic, 2-ketogluconic, arabonic, erythronic, and oxalic acids; arabinose, erythrose, and carbon dioxide. Gluconic acid is the primary oxidation product of glucose which indicates that the position of attack is principally at the anomeric carbon-hydrogen bond.

The oxidation of glucose to products containing fewer carbon atoms is thought to proceed mainly in a one-carbon, stepwise manner.

SUGGESTIONS FOR FUTURE WORK

A number of topics for future investigation have become apparent as a result of this work. A study of the formation of 5-hydroxymethylfurfural and its subsequent reactions under the experimental conditions of this work would be of great interest. It appears that these reactions are important competing reactions with respect to those by which glucose is oxidized.

Another subject which merits further work is the volatile components of the reaction. Although it was concluded that carbon dioxide was the major volatile product, this conclusion could not be established unambiguously.

This work appears to be the first study of the uncatalyzed, nonphoto-initiated oxidation of a simple carbohydrate in aqueous solution by molecular oxygen. It would be of interest to learn if this reaction is of order 1.5 with respect to other carbohydrates, because this reaction order indicates direct initiation of the reaction by oxygen.

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APPENDIX I

ISOTOPE DILUTION ANALYSIS

GENERAL TECHNIQUES

The oxidation of glucose in aqueous solution by molecular oxygen proceeds slowly and, in the early stages of the reaction, the concentrations of products are quite low. In order to determine quantitatively the amounts of the reaction products studied, the analyses were made utilizing the technique of isotope dilution.

Isotope dilution analysis may be performed by two methods. The reactant to be studied may be labeled with a radioactive atom. After the reaction has been conducted, the analysis for a particular component is carried out by the addition of a known amount of the inactive, i.e., unlabeled, component to the reaction mixture. The component is then isolated by any convenient procedure and, after purification, its reduced activity is measured. From this value, the amount of inactive component added during the dilution step, and the original activity of the starting material, the amount of labeled component in the reaction mixture may be calculated.

In the second method of analysis, the reactant is not radioactive. In order to use the technique in this manner, labeled forms of the various components for which analyses are to be made are necessary for use in the dilution step. The remainder of the procedure is the same as described in the preceding paragraph.

The first method was used in the present study to circumvent the difficulty of obtaining the various labeled products required in the second procedure. The technique was found to be accurate to within 2%.

CALCULATIONS

The calculations required in isotope dilution analysis are straightforward and are outlined by Calvin, et al. (98). For the method used in this study, (i.e., isotope dilution of active material with inactive material), the following relationship may be derived.

Let A = specific activity of starting material a.

x = amount of active component to be determined after reaction.

b = amount of inactive component for isotope dilution.

After the reaction has been conducted, the total activity of Component x in the reaction mixture is xA. If Component b is added to the mixture, the total amount of this material is x + b. The reaction mixture is then analyzed and a small amount of the desired material is isolated. After purification its reduced specific activity is determined to be C. The total activity of the diluted component is (x + b)C. Since there was no loss of radioactivity in the dilution step, the total activities must be equal:

$$xA = (x + b)C \qquad (61).$$

Solving Equation (61) for x:

$$x = bC/(A - C) \qquad (62).$$

From Equation (62), the amount of a component, x, in a reaction mixture can be readily calculated if the amount of the inactive component added to the mixture and the specific activities of the starting material and the diluted component are known. The only steps in this technique which are quantitative are the dilution step and the specific activity measurements.

In order to illustrate the application of Equation (62), the data for the determination of the unreacted glucose in Oxidation 30 (72 hours at 125°C.) are

summarized below. In the analysis, a 25-ml. aliquot (i.e., one-quarter) of Solution 30 was used.

Initial specific activity of glucose, $\underline{A}$		19,272 dis./min./mg. active carbon
Amount of inactive glucose added, $\underline{b}$		2.0000 g.
Final specific activity of GPA*, $\underline{C}$		
9th recrystallization,	Sample 1	3135 dis./min./mg. active carbon
	Sample 2	3116 dis./min./mg. active carbon
10th recrystallization,	Sample 1	3054 dis./min./mg. active carbon
	Sample 2	3116 dis./min./mg. active carbon
	Average	3105 dis./min./mg. active carbon

Unreacted glucose/25 ml. solution, $\underline{x} = bC/(A - C)$

$= 0.3842$ g.

Total unreacted glucose, $4\underline{x}$ $= 1.5368$ g.

*GPA represents β-D-glucose pentaacetate.

APPENDIX II

PURIFICATION OF INACTIVE COMPOUNDS FOR ISOTOPE DILUTION

GLUCOSE

Inactive reagent-grade anhydrous D-glucose was recrystallized by the general procedure of Isbell, et al. (85) as described on p. 68 for the purification of the labeled material. The melting point of the purified product was 144.5-147°C. The equilibrium specific rotation, $[\alpha]_D^{23}$, was + 52.7° (c 4, water). The respective literature values (85) are 146°C. and + 52.7°.

GLUCONIC ACID

D-Glucono-1,5-lactone was used as the inactive constituent during the isotope dilution analyses for gluconic acid. Reagent-grade glucono-1,5-lactone was further purified by recrystallization from 2-methoxyethanol according to the general procedure of Isbell and Frush (99).

Twenty-five grams of the lactone were added to 100 ml. of dioxane at 60-70°C. Water was then added slowly to dissolve the lactone. After the solution had been cooled in an ice-water bath, five volumes of 2-methoxyethanol, based on the amount of water used, were added and the solution refrigerated for five days. The crystalline product was filtered and dried in vacuo: m.p. 152.5-155°C.; $[\alpha]_D^{22}$ = + 65.2° → 9.8° (24 hr.; c 5, water). The literature values are (99): m.p. 150-152°C.; $[\alpha]_D^{20}$ = + 66 → +9° (24 hr.; c 5, water).

ARABINOSE

β-D-Arabinose was purified by the method of Whistler and BeMiller (100). Sixty-five grams of the crude material was dissolved in water and decolorized with Darco G-60 vegetable charcoal. The colorless solution was concentrated in vacuo

to approximately 100 ml., cooled, and 285 ml. hot methanol added. The solution was refrigerated for 48 hours, after which 85 ml. of absolute ethanol were added. The solution was refrigerated an additional 48 hours. The crystalline product was filtered and dried *in vacuo* at 60°C. A second recrystallization in the same manner yielded pure β-D-arabinose: m.p. 159-162°C.; $[\alpha]_D^{22}$ = -104.3° (*c* 2, water). Reported literature values are (100): m.p. 158.5-160°C.; $[\alpha]_D^{25}$ = 105° ± 3° (*c* 2, water).

ARABONIC ACID

The D-arabono-1,4-lactone used for isotope dilution was purified by the procedure of Isbell and Frush (101) for the preparation of this compound from its calcium salt.

Ten grams of crude arabono-1,4-lactone were dissolved in water and filtered to remove the residual calcium oxalate. The solution was then decolorized with Darco G-60 vegetable charcoal. Five drops of concentrated hydrochloric acid were added to the solution which was subsequently concentrated *in vacuo* to 13 ml. After the addition of 19 ml. of *n*-butanol, the solution was again concentrated to 13 ml. The procedure was repeated a second time, after which the sirup was seeded with the lactone. After three hours at room temperature, the crystalline product was filtered, washed with *n*-butanol, and dried *in vacuo* at 30°C. The melting point was 95.5-98.5°C.; literature value: 95-98°C. (101). The specific rotation, $[\alpha]_D^{23}$, was -70.9° (water); literature value: $[\alpha]_D^{20}$ = -71.6° (water).

OXALIC ACID

Anhydrous oxalic acid was prepared from oxalic acid dihydrate by the procedure of Bowden (102). One hundred grams of oxalic acid dihydrate were spread in a layer 3-4 mm. deep in an enameled tray which had been previously heated to 99°C.

The tray was then replaced in the oven for six hours. After the tray had been removed from the oven, the product was quickly crushed and transferred to a stoppered bottle. The anhydrous acid melted at 187-187.5°C.; literature value: 189.5°C. (102).

A known weight of anhydrous oxalic acid was transferred to a 100-ml. volumetric flask and diluted to volume with water. The purity of the acid, determined in triplicate by titration of 25-ml. aliquots of the solution with standardized sodium hydroxide solution, was found to be 96.7%.

APPENDIX III

SEPARATION OF NEUTRAL AND ACIDIC FRACTIONS

The amounts of unreacted glucose in the reaction solutions were quite large compared with the amounts of gluconic and arabonic acids formed during the reaction. From preliminary work it was found that the phenylhydrazide derivatives of the acids were more readily prepared and isolated if the aliquot of solution used for the determination was separated into a neutral and an acidic fraction. Additionally, by this procedure the same aliquot of solution could be used for the concurrent determination of a neutral and an acidic component.

The fractionation was accomplished with ion-exchange resins packed in glass columns. Amberlite IR-120 and IR-45 resins were used as the cation and anion-exchange resins, respectively. The column arrangements are shown in Fig. 9. Each column was 2 cm. in diameter, 25 cm. in length, and contained 150 meq. of resin. The resin was supported on glass wool. The use of stopcocks provided an accurate method to control the flow rates through the columns.

The following description of the separation of arabinose and arabonic acid illustrates the manner in which the columns were used.

A 25-ml. aliquot of a reaction solution was transferred to a 150-ml. beaker followed by the addition of known amounts of inactive arabinose and arabono-1,4-lactone. The solution was placed in a constant temperature bath at 50°C. and titrated with 1N sodium hydroxide to pH 10. This procedure converted the acids to their sodium salts and facilitated equilibration of the active acid in the reaction solution and the inactive lactone added during the isotope dilution step. After cooling, the solution was diluted with water to a concentration of approximately 0.1N based on the amount of alkali required during the titration.

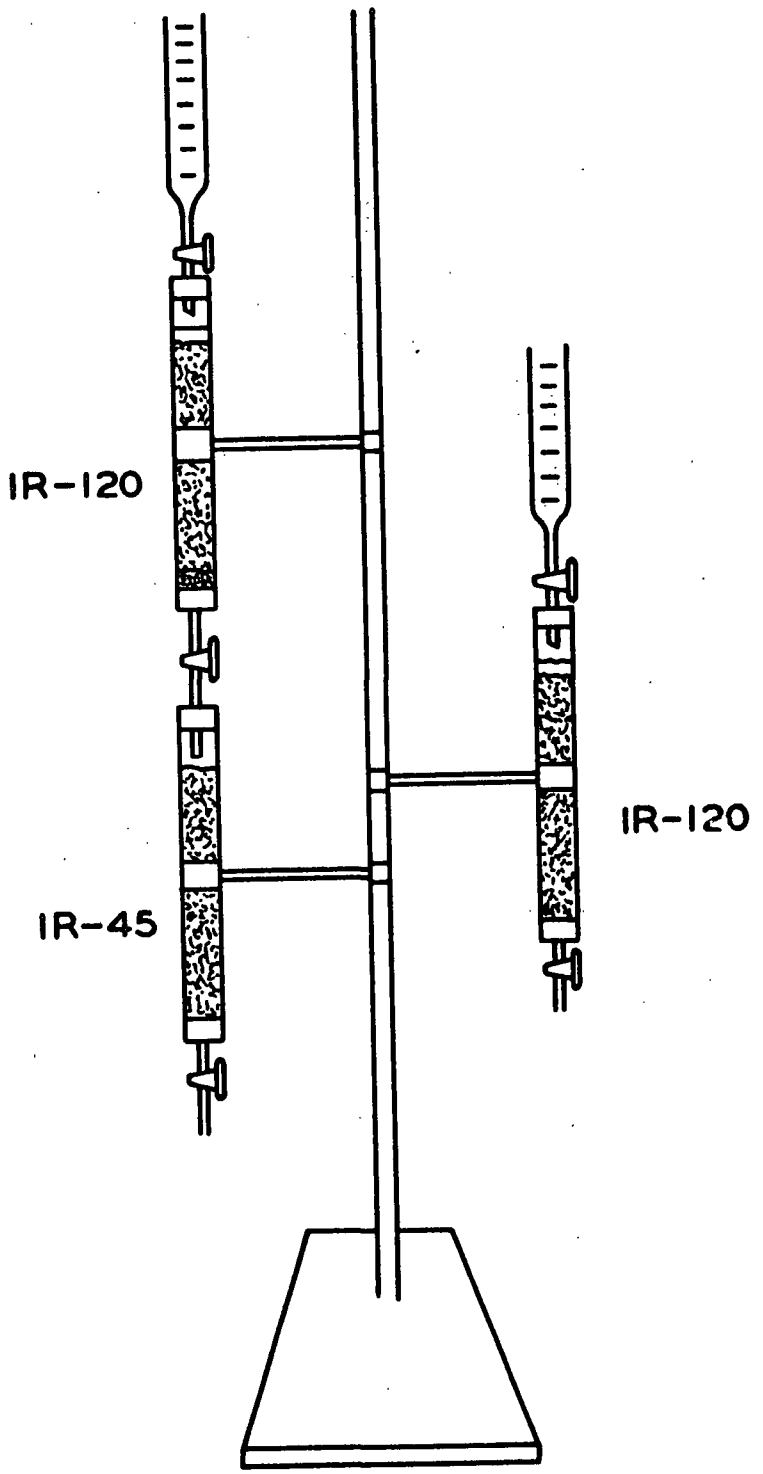

Figure 9. Ion-Exchange Columns for Separation of Neutral and Acidic Fractions

The solution was passed through the IR-120 (H^+) column directly into the IR-45 (OH^-) column. The flow rate was 4 ml./min. The free acids generated in the first column were removed in the second column. The eluate from the IR-45 column plus the water washings contained the neutral fraction. Arabinose was isolated from this fraction as its diphenylhydrazone derivative.

The acidic fraction was eluted from the IR-45 column with 1N ammonium hydroxide. After the eluate had been concentrated *in vacuo* to remove the excess ammonia, it was diluted to a concentration of approximately 0.1N. The free acids were regenerated by passing the diluted solution through the second IR-120(H^+) column at a flow rate of 4 ml./min. The eluate and water washings constituted the acidic fraction from which arabonic acid was isolated as its phenylhydrazide derivative.

The IR-120 and IR-45 columns were regenerated with 1N sulfuric acid and 1N sodium hydroxide, respectively, prior to the next separation.

APPENDIX IV

QUALITATIVE CHROMATOGRAPHY

Descending paper chromatography was used in the early phases of the product analysis work to investigate the nonvolatile oxidation products of glucose. They were identified by comparing their mobilities in several developers with those of known compounds. The chromatograms were run on Whatman No. 1 paper exclusively.

The following developers were used in this work:

A. Ethyl acetate: acetic acid: water (9:2:2 v/v).

B. n-Butanol: formic acid: water (5:1:5 v/v).

C. Ethyl acetate: pyridine: water (8:2:1 v/v).

Generally, the most useful systems for the determination of the acidic components were A and B. Gluconic, arabonic, and erythronic acids were well-resolved in these developers. System C was used to verify the identity of the neutral components arabinose and erythrose, since the acidic materials were nearly immobile.

The following reagents were used for spot detection:

(1). Silver nitrate – sodium hydroxide – sodium thiosulfate (103).

(2). p-Anisidine hydrochloride (104).

(3). Aniline phthalate (105).

Reagent (1), which was employed as a three-stage dip, detects a wide variety of compounds. It was generally employed in conjunction with Developers A and B. Reagents (2) and (3), which react with reducing groups, were used to identify 2-ketogluconic acid, arabinose, and erythrose on chromatograms run in systems B and C.

Approximate R_g values for the products identified in this work are reported in Table XIV.

TABLE XIV

APPROXIMATE R_g VALUES OF REACTION PRODUCTS

Compound	Developer A	B	C
Glucose	1.00	1.00	1.00
Gluconic Acid	1.33, 3.31[a]	1.38, 4.75[a]	0.07, 5.53[a]
2-Ketogluconic acid	--	1.42	0.05
Arabinose	1.61	1.77	1.57
Arabonic acid	1.65, 3.63[a]	2.32, 5.54[a]	0.09, 5.94[a]
Erythrose	2.95	4.94	3.86
Erythronic acid	2.25, --[a]	3.54, 6.36[a]	0.22, 6.10[a]

[a]Lactone.